Dietary and Genetic Risk Factors for Parkinson's Disease

DIETARY AND GENETIC RISK FACTORS FOR PARKINSON'S DISEASE

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Abstract

Parkinson's disease (PD) is the second most common neurodegenerative disease. Motor symptoms typically do not manifest until significant neuronal loss has already occurred, highlighting the need for early detection and prevention. In this dissertation, we sought to improve our understanding of PD epidemiology by studying associations between potential modifiable risk factors, including antioxidant vitamins, dairy products, and urate, and PD risk. We conducted prospective analyses within three large cohort studies: the Nurses' Health Study, the Health Professionals Follow-up Study, and the Cancer Prevention Study II Nutrition Cohort. Across all analyses, PD cases were identified via biennial questionnaires and confirmed through medical record review by neurologists specializing in movement disorders.

In our first two aims, we used Cox proportional hazards models to calculate relative risks of PD according to cumulative average intakes of foods and nutrients of interest. In aim 1, we found no associations between intake of vitamin E, vitamin C, or carotenoids and risk of PD. In our second aim, we found that low fat dairy intake was associated with increased PD risk, and that this association appeared to be driven by an increased risk of PD associated with skim and low-fat milk intake. The results of a meta-analysis including previously conducted prospective investigations of milk intake and PD risk suggested a relative risk of PD comparing extreme milk intake levels of 1.80 (95% CI 1.44-2.25). In our third aim, although a large body of research suggests that higher urate levels could be protective against PD risk and progression, we found that genetic variants in the *SLC2A9* gene that influence circulating urate levels were not associated with risk of PD.

Our analyses suggest that while antioxidant vitamins are unlikely to alter PD risk, dairy products may represent an important modifiable PD risk factor. Whether dairy products also alter rates of PD progression or conversion from premotor PD to clinical PD are important, answerable questions. Finally, the results of our third analysis suggest that genetic variants associated with plasma urate levels are not associated with PD risk; however, larger studies are needed to confirm these results.

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Chapter 1 Intake of Antioxidant Vitamins and Risk of Parkinson's Disease

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ABSTRACT

Background: Oxidative stress is proposed to be one of the mechanisms leading to neurodegeneration in Parkinson's disease (PD). However, previous epidemiologic studies investigating associations between antioxidant vitamins, such as vitamin E, vitamin C, and carotenoids, and PD risk have produced inconsistent results.

Objective: To prospectively examine associations between intakes of vitamins E and C and carotenoids and risk of PD.

Methods: PD cases were identified in two large cohorts: the Nurses' Health Study, comprising 81,479 women followed for 28 years, and the Health Professionals Follow-up Study, comprising 49,750 men followed for 24 years. Members of both cohorts completed detailed, validated semi-quantitative food frequency questionnaires at baseline and every four years thereafter.

Results: 1036 PD cases were identified. Dietary intakes of vitamin E and carotenoids were not associated with altered PD risk; the multivariable-adjusted relative risk (95% confidence interval) for the highest versus lowest quintiles of intake were 0.93 (0.75, 1.14) and 0.97 (0.69, 1.37), respectively. Dietary vitamin C intake was significantly associated with reduced PD risk (RR 0.81; CI 0.65-1.01; *p* trend 0.01); however, this result was no longer significant in a four-year lag analysis. For vitamins E and C, intake from foods and supplements combined were unrelated to PD risk. We examined PD risk in relation to intake of specific carotenoids, including beta-carotene, alpha-carotene, lutein, lycopene, and beta-cryptoxanthin—no significant associations were found.

Conclusions: Our results do not support the hypothesis that intake of antioxidant vitamins at levels commonly consumed in the US reduces the risk of PD.

INTRODUCTION

Oxidative stress is proposed to be a possible cause of neurodegeneration in Parkinson's Disease (PD)[1-2]. Because dietary antioxidants (e.g., vitamins E, C, and carotenoids) can protect against oxidative damage[3], it has been hypothesized that intake of these nutrients could reduce the risk of PD[4]. However, epidemiologic evidence in this area has been inconsistent[5-6]. A meta-analysis conducted in 2005 suggested that intake of vitamin E could decrease the risk of developing PD, while vitamin C and beta carotene did not show a significant association with PD[7]. However, several of the studies cited in this analysis were not prospective and thus recall bias could have affected the results.

To address this issue, we prospectively examined associations between intake of vitamin E, vitamin C, carotenoids, and risk of PD in the Nurses' Health Study (NHS) and the Health Professionals Follow-Up Study (HPFS). Results from these cohorts examining this question have been published previously[8]; however, we now present data after an additional 12 years of follow-up and over 1000 cases of PD. We expect the additional power in this analysis to lead to more definitive results.

METHODS

Study population

The NHS cohort was established in 1976 when 121,700 female registered nurses aged 30 to 55 years completed a mailed questionnaire regarding their medical histories and health-related behaviors. The HPFS cohort was established in 1986 when 51,529 male health professionals aged 40 to 75 years responded to a similar questionnaire. For both cohorts, follow-up questionnaires have been sent every two years to update information on potential risk factors and newly diagnosed diseases. For this analysis, the 81,757 nurses who returned a 1984 food frequency questionnaire regarding usual dietary intake were the baseline population in the NHS, while 49,934 health professionals who returned a 1986

food frequency questionnaire were the baseline population in the HPFS. Individuals were excluded if they reported implausible total energy intake at baseline (<660 or >3500 kcal/day for women and <800 or >4200 kcal/day for men), had a previous diagnosis of PD, or had missing baseline dietary information. This left a total of 81,479 women and 49,750 men for the analysis. This study was approved by the Human Research Committees at the Brigham and Women's Hospital and the Harvard T. H. Chan School of Public Health.

Dietary assessment and other covariates

Participants completed food frequency questionnaires that assessed how often over the past year they typically consumed a commonly used portion of each food. There were nine possible responses, ranging from "never" to "six or more times per day". Nutrient intakes were then computed by multiplying the frequency response by the nutrient content of the specified portion size, using data from the US Department of Agriculture and from manufacturers. Detailed information on supplemental vitamin use was also collected. For NHS diet was assessed in 1984 (baseline), 1986, and every four years thereafter. For HPFS, diet was assessed in 1986 (baseline) and every four years thereafter. Validation studies for vitamin C, vitamin E and carotenoid intake were conducted in subsets of participants for both cohorts. In NHS, the correlations between intakes measured by the FFQ and by the average of four one-week diet records were 0.76 for total vitamin C (i.e., from foods and supplements), and 0.64 for dietary vitamin C (i.e., from foods only)[9]. In HPFS, the correlations between participants' FFQ and the average of two one-week diet records were 0.92 for total vitamin C, 0.77 for dietary vitamin C, 0.92 for total vitamin E, and 0.42 for dietary vitamin E[10]. The correlation between total vitamin E measured by FFQ and plasma α-tocopheral levels was 0.41 in NHS and 0.51 in HPFS[11]. Among nonsmoking women in NHS, adjusted correlations between plasma carotenoid markers and dietary measures from the FFQ were 0.48 for alpha carotene, 0.27 for beta-carotene, 0.32 for beta-cryptoxanthin, 0.27 for lutein, and 0.21 for

lycopene. Among nonsmoking men in HPFS, these correlations were 0.47 for alpha-carotene, 0.35 for beta-carotene, 0.43 for beta-cryptoxanthin, 0.40 for lutein, and 0.47 for lycopene. Importantly, these correlations between intake and plasma levels likely underestimate the validity of long-term dietary intakes as assessed by the FFQ because they are not corrected for error in estimating plasma levels or for short term variations in plasma levels[12]. Information on other covariates of interest was also collected via self-report questionnaire for both cohorts.

Ascertainment of PD cases

PD cases were identified via biennial self-report questionnaire in which cohort members were asked to report new diagnosis of illnesses. We then contacted treating neurologists of the self-reported cases who were asked to confirm the diagnosis or send a copy of the patients' medical records. Prior to 2003, cases were considered confirmed if the treating neurologist or internist considered the diagnosis of PD definite or probable, the medical record included a final diagnosis of PD by a neurologist, or the medical record indicated the presence of at least two of three cardinal signs of PD (resting tremor, rigidity, bradykinesia) in the absence of evidence for other diagnoses. For cases of PD reported since 2003, the above procedure was used with the exception that medical records were requested from all cases and were reviewed by a neurologist specializing in movement disorders. If the determination of the movement disorders specialist conflicted with that of the neurologist, the decision of the movement disorders specialist was used. In a validation study within a subset of confirmed cases, updated medical records were reviewed several years after case ascertainment and showed that 96 out of 100 cases who had been considered definite or probable were confirmed to have PD, while four were thought to be uncertain cases. Only confirmed cases were included in this analysis.

Statistical analysis

Subjects contributed person-years of observation from the age in months at the date of returning the baseline food frequency questionnaire to the age in months at the date of PD diagnosis, death, last completed questionnaire, or end of follow-up (June 2010 for NHS and January 2010 for HPFS), whichever came first. The analysis was stratified by age in months at start of follow-up and calendar year of current questionnaire cycle (to control as finely as possible for confounding by age, calendar time, and any potential interactions between these variables). For nutrient analyses, PD incidence was related to cumulative updated average intake from all available dietary questionnaires up to the start of each two-year follow-up period, categorized by cohort-specific quintile of intake[13]. Secondary analyses were conducted using baseline nutrient intake levels. Separate models were fit for each exposure of interest: dietary vitamin E, dietary vitamin C, and dietary carotenoids. In addition, total vitamin E and vitamin C intake (including supplements) were investigated. Nutrients were adjusted for total energy using the residual method so that they would be uncorrelated with total energy intake[14]. Age-adjusted and multivariate-adjusted hazard ratios were calculated for each exposure using Cox proportional hazards models. Multivariate models simultaneously adjusted for pack years of smoking (never smoker, 1 to <5, 5 to <10, 10 to <15, ≥15), coffee intake (non, <1, 1-3, 4-5, or ≥6 cups/day), body mass index (BMI, <21, 21 to <25, 25 to <30, 30 to <35, or ≥35 kg/m^2), physical activity (in quintiles), alcohol intake (none, 0.1 to 4.9, 5.0 to 9.9, 10.0 to 14.9, or ≥15 g/day for women and none, 0.1 to 9.9, 10.0 to 19.9, 20.0 to 29.9, or ≥30 g/day for men), and total energy intake (in quintiles). Follow-up analyses separately examined each of five major carotenoids: lutein, lycopene, alpha-carotene, beta-carotene, and beta-cryptoxanthin. Results from both cohorts were pooled using random-effects methods. We conducted a lagged analysis excluding the first four years of follow-up in each cohort to address the possibility that participants could be experiencing PD symptoms at the time of questionnaire completion. Finally, we evaluated whether the association between vitamin E intake and

PD risk was modified by dairy (high vs lower, based on median value), BMI (high vs lower), or the dietary urate index (comprised of intake of fructose, vitamin C, alcohol, and dairy protein; high vs lower)[15] by including interaction terms in the models. We similarly evaluated potential effect modification between all exposures and caffeine intake (high vs lower). All statistical analyses were conducted using SAS (SAS Institute, Cary, NC).

RESULTS

A total of 1036 cases (554 in HPFS and 482 in NHS) were observed over the follow-up period. Baseline characteristics of each cohort are presented in Table 1.1. Among women in the NHS and among men in the HPFS, neither total vitamin E (age-adjusted *p* trend = 0.68; multivariable-adjusted *p* trend = 0.82) nor total vitamin C (age-adjusted *p* trend = 0.78; multivariable-adjusted *p* trend = 0.93) were significantly associated with risk of PD (Figure 1.1). Dietary vitamin E was also unassociated with risk of PD (age-adjusted *p* trend = 0.62; multivariable-adjusted *p* trend= 0.93). Dietary vitamin C was inversely associated with PD risk (pooled RR comparing highest intake quintile to lowest quintile = 0.81; 95% CI 0.65-1.01; *p* trend 0.01); however, this result was no longer significant in a four-year lagged analysis (pooled RR 0.86; 95% CI 0.58-1.28; *p* trend 0.30). We further examined associations for dietary vitamins C and E after excluding supplement users and obtained similar results: the pooled multivariable-adjusted relative risk comparing highest to lowest intake quintiles for dietary vitamin C was 0.74 (95% CI, 0.55-0.98; *p* trend = 0.06) and for dietary vitamin E was 0.80 (95% CI, 0.62-1.02; *p* trend = 0.39). Again, the results for dietary vitamin C were non-significant in a lagged analysis (pooled RR 0.85; 95% CI 0.51-1.42; *p* trend = 0.33).

Table 1.1 Age-adjusted characteristics of the study population at baseline by quintile of total vitamin intake

	Quartile of Vitamin E Intake				
	1	2	3	4	5
Health Professionals Follow-up Study, 1986-2010	n=10003	n=9701	n=9890	n=9794	n=9834
Age, years*	53.1(9.6)	53.7(9.7)	54.8(9.8)	55.4(10.0)	56.9(9.6)
Body mass index, kg/m^2	25.7(3.3)	25.7(3.3)	25.7(3.6)	25.4(3.3)	25.2(3.2)
Current smoker, %	13	10	8	9	9
Past smoker, %	42	44	44	46	44
Caucasian, %	95	96	95	96	96
Activity, met-h/week	15.4(22.8)	17.5(24.4)	19.6(26.9)	20.0(26.6)	21.3(29.3)
Coffee, servings/day	1.5(1.7)	1.4(1.6)	1.3(1.6)	1.3(1.5)	1.1(1.5)
Alcohol, g/day	13.6(18.4)	10.7(14.5)	10.4(14.0)	10.9(14.7)	11.3(15.3)
Total energy intake, kcal/day	1730(529)	1980(575)	2171(634)	2086(648)	1961(614)
Nurses' Health Study, 1984-2008					
Age, years*	n=15553	n=16842	n=16150	n=16072	n=16147
	50.1(7.2)	50.4(7.2)	50.9(7.1)	51.3(7.3)	52.1(6.9)
Body mass index, kg/m²	25.8(5.0)	26.1(5.1)	26.3(5.1)	25.7(4.8)	25.4(4.7)
Current smoker, %	32	25	22	22	21
Past smoker, %	27	30	33	34	35
Caucasian, %	98	98	97	98	98
Activity, met-h/week	10.7(16.8)	12.6(18.7)	14.8(21.2)	15.5(22.1)	16.7(24.2)
Coffee, servings/day	1.9(1.8)	1.9(1.8)	1.8(1.8)	1.7(1.7)	1.6(1.7)
Alcohol, g/day	7.8(13.4)	6.5(10.4)	6.5(10.3)	6.8(11.0)	6.9(11.0)
Total energy intake, kcal/day	1495(447)	1743(478)	1935(534)	1839(545)	1702(528)

Values are means(SD) or percentages and are standardized to the age distribution of the study population.

[a] Metabolic equivalents from recreational and leisure-time activities

[*] Value is not age-adjusted

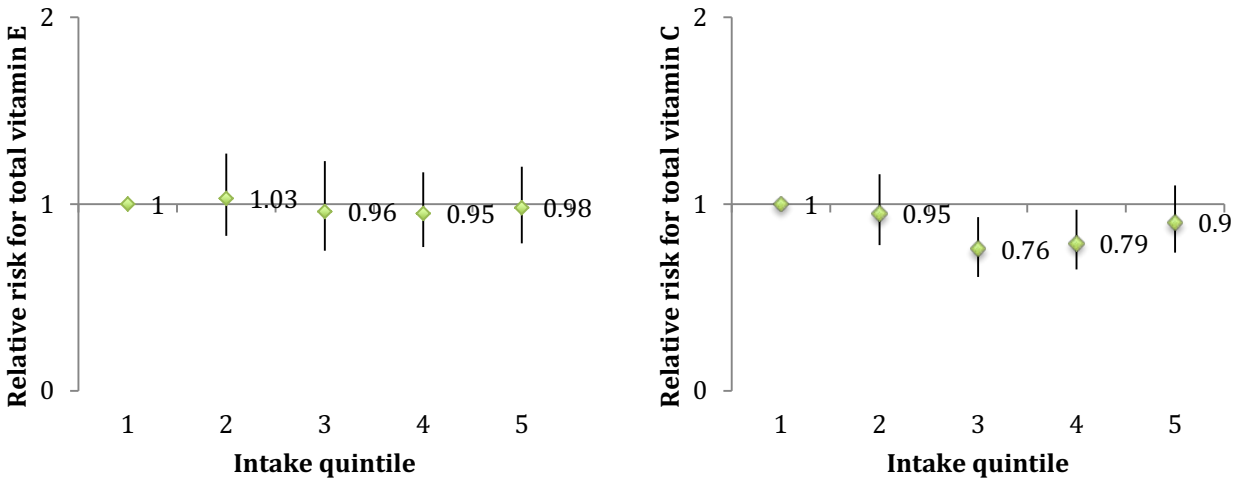
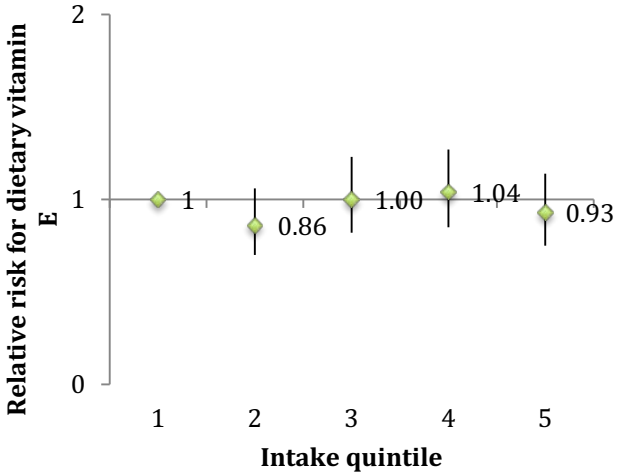


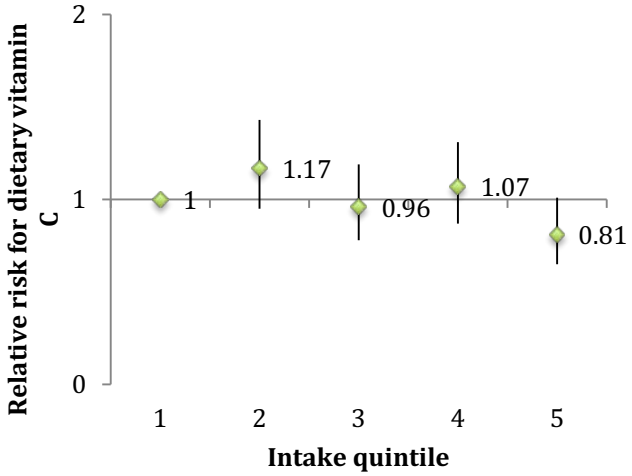
Figure 1.1 Associations of total vitamins E and C with PD according to intake quintiles, using cumulative average intake levels and adjusted for pack years of smoking, coffee intake, body mass index, physical activity, alcohol intake, and total energy intake. Median intake levels for each quintile of total vitamin E were 6.0, 7.6, 9.3, 14.6, and 176.8 IU/day among women and 7.6, 9.6, 11.8, 18.8, and 193.6 IU/day among men at baseline. Median intake levels for each quintile of total vitamin C were 79, 130, 183, 302, and 825 mg/day among women and 95, 157, 228, 403, and 1159 mg/day among men at baseline.

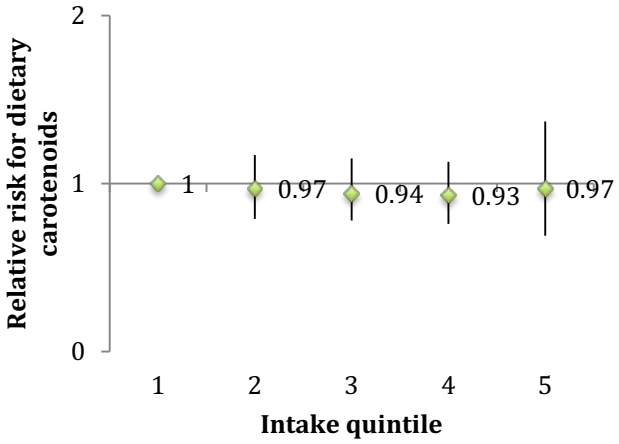
For dietary carotenoids, we also found no significant association with the risk of PD (age-adjusted *p* trend = 0.83; multivariable-adjusted *p* = 0.82). We also examined the association for intake of individual carotenoids, including α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein. None were significantly associated with risk of PD (Figure 1.2). Our main results were similar when we used baseline nutrient intake rather than cumulative average intake, as well as when restricting to non-smokers and to definite cases of PD. As a follow-up analysis in order to investigate whether risk is only affected by very high or very low doses, we calculated relative risks comparing individuals with vitamin C intakes above versus below 1000 mg/day (pooled RR = 1.13 (95% CI, 0.91-1.39; *p* = 0.28), as well as comparing individuals with intakes above versus below 100 mg/day (pooled RR=1.09 (95% CI, 0.81-1.47; *p* trend = 0.56). In the case of vitamin E, we conducted similar analyses comparing individuals above versus below 200 IU/day (pooled RR = 1.07 (95% CI, 0.86-1.33; *p* = 0.53) and 10 IU/day (pooled RR = 1.08

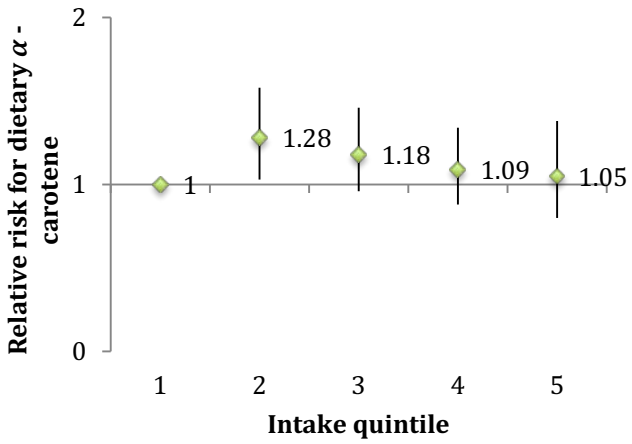
(95% CI, 0.91-1.27; *p* = 0.38), and in the case of individual and total carotenoids, comparing individuals with intakes levels above versus below the 90th and 10th percentiles of the cohort-specific distributions. For total carotenoids, the pooled RR comparing individuals above versus below the 90th percentile was 0.89 (95% CI, 0.58-1.37; *p* = 0.60) and the pooled RR comparing individuals above versus below the 10th percentile was 0.95 (95% CI, 0.77-1.18; *p* = 0.65). Analyses for specific carotenoids also did not yield significant results (data not shown). Results were not substantially affected by conducting a lag analysis or after further adjustment for flavonoids, dairy intake, the Alternate Health Eating Index (comprised of fruit, vegetables, nuts and soy, and other dietary components)[16], the dietary urate index, and NSAIDs. We also investigated whether duration of supplemental vitamin C or E use was associated with risk of PD. We found no significant associations for duration of supplemental vitamin E use (compared with non-users, pooled multivariable-adjusted RR among users for ≥ 15 years = 0.96 [95% CI, .76-1.20; *p* trend = 0.59]) or duration of supplemental vitamin C use (RR for ≥ 15 years of use = 0.87 [95% CI, 0.73-1.07; *p* trend = 0.14) in men and women. In order to investigate whether supplemental vitamin C or E intake might yield benefits only among individuals with low intake of dietary vitamin C, vitamin E, and carotenoids, we created an antioxidant score by summing the intake quintile (0-4) of dietary vitamin C, vitamin E, and carotenoids for each participant, such that an individual in the lowest quintile of each nutrient would obtain a score of 0, and an individual in the highest quintile of each nutrient would obtain a score of 12. We then examined the effect of supplemental vitamin C and E intake only among individuals with antioxidant scores below the median value. Consistent with other analyses, we found no evidence of a beneficial effect of supplemental vitamin C or E: comparing individuals in the highest intake category of supplemental vitamin C (≥ 700 mg/day) to individuals who did not take supplemental vitamin C, the pooled RR was 0.84 (95% CI, 0.60-1.18; *p* trend = 0.24). Comparing individuals in the highest intake category of supplemental vitamin E (≥ 600 IU/day) to individuals who did not take supplemental vitamin E, the pooled RR was 0.87 (95% CI, 0.54-1.43; *p* trend = 0.22). Finally, we observed

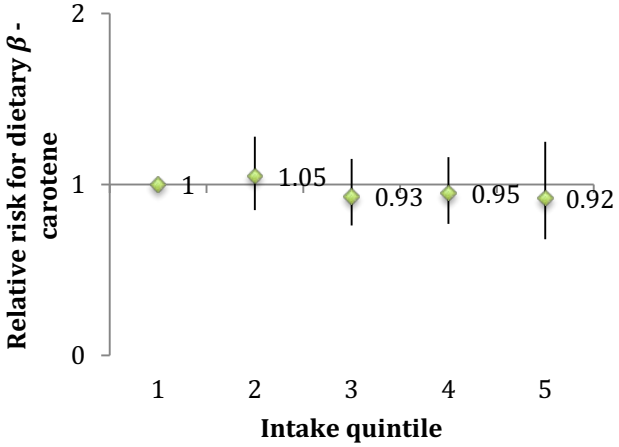
no significant interactions between caffeine and any of the exposures, or between vitamin E and BMI, dairy intake, or dietary urate index ($p > 0.05$ for all).

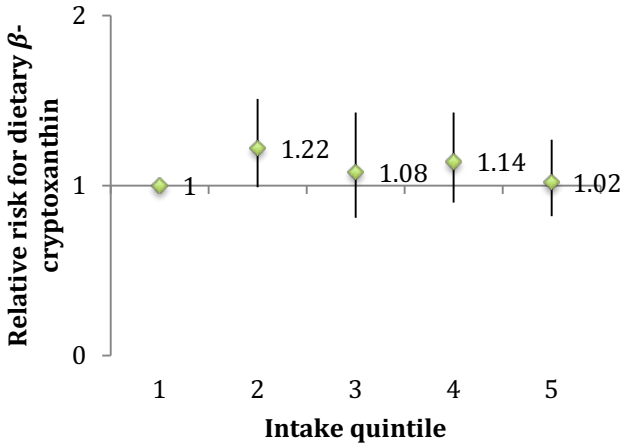












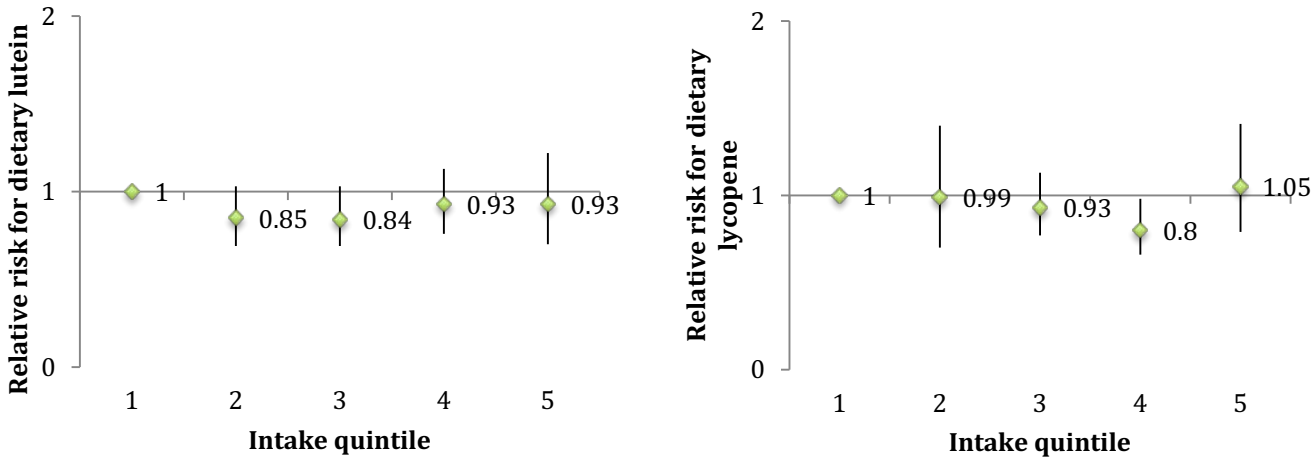


Figure 1.2 Associations of dietary vitamin E, vitamin C and carotenoids with PD according to intake quintiles, using cumulative average intake levels and adjusted for pack years of smoking, coffee intake, body mass index, physical activity, alcohol intake, and total energy intake. Median intake levels for each quintile of nutrients at baseline were as follows: for dietary vitamin E 5.8, 6.8, 7.6, 8.5, and 10.2 IU/day among women, and 7.3, 8.7, 9.8, 11.1, and 13.7 IU/day among men; for dietary vitamin C 67, 101, 128, 158, and 215 mg/day among women, and 78, 121, 154, 193, and 267 mg/day among men; for dietary carotenoids 7502, 10561, 13238, 16567, and 23008 mg/day among women and 7902, 11602, 14854, 18947, and 26940 mg/day among men; for *β*-cryptoxanthin 62, 115, 170, 228, and 328 mg/day among women, and 66, 126, 188, 256, and 384 mg/day among men; for *α*-carotene 248, 407, 541, 837, and 1661 mg/day among women, and 269, 458, 617, 995, and 1933 mg/day among men; for *β*-carotene 1712, 2616, 3516, 4759, and 7292 mg/day among women, and 1902, 2935, 3958, 5383, and 8238 mg/day among men; for lutein 1137, 1903, 2576, 3383, 5165 mg/day among women and 1263, 2108, 2866, 3802, and 5838 mg/day among men; and for lycopene 2783, 4302, 5553, 7223, 11001 mg/day among women and 2592, 4534, 6125, 8293, and 13426 mg/day among men.

DISCUSSION

Our results suggest that greater intake of antioxidant vitamins within the range common in the U.S. population may not reduce the risk of Parkinson's disease. Strengths of this study include its prospective design, which is particularly important when studying dietary exposures as they are susceptible to reverse causation and recall bias. In addition, follow-up rates in both cohorts have been

very high, we used validated dietary assessment measures, and the large number of cases reduces the risk of false negative results.

Our study has some limitations. First, some degree of misclassification of nutrient intake is inevitable. Because of our prospective design, we expect that misclassification would be non-differential and would result in bias toward the null, which could explain our findings. However, previous research suggests that our nutrient assessment method reflects long-term intakes of study subjects reasonably well[9-12, 17-18]. In addition, we expect any errors to be reduced by the use of repeated measures in analyses using cumulative average nutrient intakes as exposures. Another limitation is the possibility that early symptoms of PD influenced dietary behaviors or questionnaire responses. However, we used first PD symptoms as our outcome rather than PD diagnosis to minimize this possibility. In addition, the results of our lagged analysis suggest that reverse causation is unlikely.

The relation between dietary antioxidant intake and PD risk has been examined in a number of cross-sectional and retrospective studies. Vitamin E was reported to be inversely associated with PD risk in two studies[19,20], although in one study this association was only significant among women[20]. Another cross-sectional study reported an inverse association between PD risk and vitamin C and a non-significant inverse association with beta-carotene[5]. However, positive associations with PD risk have been reported for lutein[21] as well as vitamin C and carotenoids[22]. In other case-control studies no associations were found between PD and vitamin C[19,23,20,24,25], vitamin E[5,23,24,25], and specific carotenoids[19,20,24,25]. The results of all these cross-sectional or case-control investigations, however, are difficult to interpret, because of the potential impact of recall and selection bias.

While few prospective studies have been conducted examining these associations, results have tended to be null. Investigators from the Honolulu Heart Study Cohort assessed diet by combining data from 24-hour recall and FFQs and, after identifying 84 incident cases over up to 30 years of follow-up,

found no association between vitamin E and risk of PD[26]. Results from another study in which 395 cases were identified over 17 years of follow-up showed no association between vitamin C measured using an FFQ and risk of PD[27]. In a Chinese cohort including 157 incident PD cases over 12 years of follow-up, vitamin E intake was associated with lower PD risk; however, no association was found for vitamin C or carotenoids[6]. In addition, in a large, double-blind, placebo-controlled clinical trial, a daily dose of 2000 IU of alpha-tocopherol, a biologically active component of vitamin E, was found to have no significant improvements on the rate of progression of PD[28]. Previous results from the NHS and HPFS showed a reduced risk of PD associated with high dietary vitamin E intake, but no association for supplemental or total vitamin E intake as well as no associations for vitamin C or carotenoids. Our updated results suggest that the previous finding for dietary vitamin E intake could have been due to chance.

It is important to note that our findings of a lack of an association between antioxidant vitamins and PD do not invalidate the role of oxidative stress in this disease. Importantly, urate has been found in prospective studies to be associated with a reduced risk of PD[29,30] and higher serum and cerebrospinal fluid concentrations of urate have been associated with slower rates of clinical decline[31,32]; these associations may be attributable to urate's powerful antioxidant properties. In addition, our group recently reported an inverse association between intake of flavonoids and risk of PD, particularly among men, in the NHS and HPFS cohorts[33]. Although a number of mechanisms could be driving this protective effect, including regulation of mitochondrial function or inflammation, flavonoids may also have antioxidant properties that could have contributed to these results.

In conclusion, our results from two large prospective cohort studies provide evidence that intake of vitamin E, vitamin C, and carotenoids, at least at the levels consumed in our cohorts, does not substantially affect the risk of PD.

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APPENDIX

Table 1.2 Relative risk of PD by quintiles of intake of vitamin E, vitamin C, and carotenoids

	Quintile					P_{trend}
	1	2	3	4	5	
Total vitamin E[a]						
Women						
Median, IU/d	6.0	7.6	9.3	14.6	176.8	
Number of cases	72	99	113	100	98	
Relative risk (95% CI)						
Age-adjusted[b]	1.0	1.13 (0.83, 1.53)	1.16 (0.86, 1.56)	0.95 (0.70, 1.29)	1.06 (0.78, 1.45)	0.71
Multivariate[c]	1.0	1.08 (0.79, 1.47)	1.09 (0.81, 1.47)	0.89 (0.65, 1.22)	1.02 (0.75, 1.39)	0.63
Men						
Median, IU/d	7.6	9.6	11.8	18.8	193.6	
Number of cases	87	94	105	140	128	
Relative risk (95% CI)						
Age-adjusted[b]	1.0	1.02 (0.76, 1.37)	0.93 (0.70, 1.24)	1.11 (0.85, 1.46)	1.06 (0.80, 1.39)	0.42
Multivariate[c]	1.0	0.98 (0.73, 1.32)	0.85 (0.63, 1.14)	1.00 (0.75, 1.31)	0.94 (0.71, 1.25)	0.93
Pooled analysis						
Multivariate[c]	1.0	1.03 (0.83, 1.27)	0.96 (0.75, 1.23)	0.95 (0.77, 1.17)	0.98 (0.79, 1.20)	0.82
Dietary vitamin E[d]						
Women						
Median, IU/d	5.8	6.8	7.6	8.5	10.2	
Number of cases	84	80	106	121	91	
Relative risk (95% CI)						
Age-adjusted[b]	1.0	0.79 (0.58, 1.07)	0.98 (0.74, 1.31)	1.10 (0.83, 1.46)	0.85 (0.63, 1.14)	0.80
Multivariate[c]	1.0	0.78 (0.57, 1.06)	0.96 (0.72, 1.28)	1.08 (0.81, 1.44)	0.83 (0.61, 1.13)	0.74
Men						
Median, IU/d	7.3	8.7	9.8	11.1	13.7	
Number of cases	92	101	119	119	123	
Relative risk (95% CI)						

Table 1.2 (Continued)

Age-adjusted[b]	1.0	1.00 (0.75, 1.33)	1.15 (0.87, 1.51)	1.10 (0.83, 1.44)	1.12 (0.85, 1.47)	0.45
Multivariate[c]	1.0	0.94 (0.71, 1.26)	1.05 (0.79, 1.38)	1.00 (0.76, 1.33)	1.02 (0.77, 1.34)	0.90
Pooled analysis						
Multivariate[c]	1.0	0.86 (0.70, 1.06)	1.00 (0.82, 1.23)	1.04 (0.85, 1.27)	0.93 (0.75, 1.14)	0.93
Total vitamin C						
Women						
Median, mg/d	79	130	183	302	825	
Number of cases	87	109	94	91	101	
Relative risk (95% CI)						
Age-adjusted[b]	1.0	1.02 (0.75, 1.33)	0.81 (0.61, 1.09)	0.77 (0.58, 1.04)	0.89 (0.67, 1.19)	0.50
Multivariate[c]	1.0	0.96 (0.72, 1.28)	0.76 (0.56, 1.02)	0.72 (0.53, 0.98)	0.85 (0.63, 1.14)	0.44
Men						
Median, mg/d	95	157	228	403	1159	
Number of cases	95	113	102	117	127	
Relative risk (95% CI)						
Age-adjusted[b]	1.0	1.01 (0.76, 1.33)	0.85 (0.64, 1.12)	0.96 (0.73, 1.26)	1.04 (0.79, 1.36)	0.44
Multivariate[c]	1.0	0.94 (0.71, 1.24)	0.76 (0.57, 1.01)	0.86 (0.65, 1.13)	0.95 (0.72, 1.24)	0.70
Pooled analysis						
Multivariate[c]	1.0	0.95 (0.78, 1.16)	0.76 (0.61, 0.93)	0.79 (0.65, 0.97)	0.90 (0.74, 1.10)	0.93
Dietary vitamin C						
Women						
Median, mg/d	67	101	128	158	215	
Number of cases	75	109	107	108	83	
Relative risk (95% CI)						
Age-adjusted[b]	1.0	1.21 (0.90, 1.63)	1.08 (0.80, 1.46)	1.05 (0.78, 1.42)	0.82 (0.60, 1.13)	0.07
Multivariate[c]	1.0	1.16 (0.86, 1.57)	1.02 (0.75, 1.38)	0.97 (0.72, 1.32)	0.77 (0.56, 1.06)	0.03
Men						
Median, mg/d	78	121	154	193	267	

Table 1.2 (Continued)

Number of cases	84	120	104	141	105	
Relative risk (95% CI)						
Age-adjusted[b]	1.0	1.25 (0.95, 1.66)	1.01 (0.75, 1.35)	1.30 (0.99, 1.71)	0.99 (0.74, 1.33)	0.75
Multivariate[c]	1.0	1.17 (0.88, 1.55)	0.92 (0.68, 1.23)	1.15 (0.87, 1.53)	0.84 (0.62, 1.14)	0.15
Pooled analysis						
Multivariate[c]	1.0	1.17 (0.95, 1.43)	0.96 (0.78, 1.19)	1.07 (0.87, 1.31)	0.81 (0.65, 1.01)	0.01
Dietary Carotenoids						
Women						
Median, mg/d	7502	10561	13238	16567	23008	
Number of cases	98	100	108	95	81	
Relative risk (95% CI)						
Age-adjusted[b]	1.0	0.94 (0.71, 1.24)	0.98 (0.74, 1.29)	0.86 (0.65, 1.14)	0.78 (0.58, 1.05)	0.07
Multivariate†	1.0	0.94 (0.71, 1.25)	0.98 (0.74, 1.29)	0.87 (0.65, 1.16)	0.81 (0.60, 1.09)	0.14
Men						
Median, mg/d	7902	11602	14854	18947	26940	
Number of cases	101	110	105	111	127	
Relative risk (95% CI)						
Age-adjusted[b]	1.0	1.04 (0.79, 1.37)	0.97 (0.74, 1.28)	1.05 (0.80, 1.37)	1.22 (0.93, 1.58)	0.15
Multivariate[c]	1.0	0.99 (0.75, 1.30)	0.91 (0.69, 1.20)	0.98 (0.74, 1.29)	1.15 (0.88, 1.50)	0.29
Pooled analysis						
Multivariate[c]	1.0	0.97 (0.79, 1.17)	0.94 (0.78, 1.15)	0.93 (0.76, 1.13)	0.97 (0.69, 1.37)	0.82

Using cumulative average intake levels.

[a] Total intake, i.e., intake from foods and supplements

[b] Adjusted for age (years)

[c] Adjusted for age, pack years of smoking, coffee intake, BMI, physical activity, alcohol intake, and total energy intake

[d] Dietary intake, i.e., intake from foods only

Table 1.3 Intake of specific carotenoids and relative risk of PD

	Quintile					P_{trend}
	1	2	3	4	5	
α-Carotene						
Women						
Median, mg/d	248	407	541	837	1661	
Cases	70	108	105	107	92	
Age-adjusted[a] HR (95% CI)	1.0	1.30 (0.96, 1.75)	1.16 (0.86, 1.58)	1.10 (0.81, 1.49)	0.96 (0.70, 1.31)	0.20
Multivariate-adjusted[b] HR (95% CI)	1.0	1.27 (0.94, 1.72)	1.12 (0.83, 1.52)	1.04 (0.77, 1.42)	0.91 (0.66, 1.25)	0.11
Men						
Median, mg/d	269	458	617	995	1933	
Cases	75	110	123	118	128	
Age-adjusted[a] HR (95% CI)	1.0	1.32 (0.98, 1.77)	1.35 (1.01, 1.81)	1.24 (0.92, 1.66)	1.33 (0.99, 1.77)	0.39
Multivariate-adjusted[b] HR (95% CI)	1.0	1.29 (0.95, 1.73)	1.24 (0.93, 1.67)	1.13 (0.84, 1.52)	1.20 (0.89, 1.61)	0.89
Pooled analysis						
Multivariate-adjusted[b] HR (95% CI)	1.0	1.28 (1.03, 1.58)	1.18 (0.96, 1.46)	1.09 (0.88, 1.34)	1.05 (0.80, 1.38)	0.47
β-Carotene						
Women						
Median, mg/d	1712	2616	3516	4759	7292	
Cases	84	112	97	100	89	
Age-adjusted[a] HR (95% CI)	1.0	1.10 (0.83, 1.46)	0.90 (0.67, 1.21)	0.89 (0.66, 1.19)	0.79 (0.59, 1.07)	0.03
Multivariate-adjusted[b] HR (95% CI)	1.0	1.09 (0.82, 1.45)	0.88 (0.65, 1.19)	0.87 (0.64, 1.17)	0.78 (0.58, 1.07)	0.03
Men						
Median, mg/d	1902	2935	3958	5383	8238	
Cases	88	103	110	123	130	
Age-adjusted[a] HR (95% CI)	1.0	1.03 (0.77, 1.37)	1.02 (0.77, 1.36)	1.09 (0.83, 1.44)	1.14 (0.86, 1.50)	0.34

Table 1.3 (Continued)

Multivariate-adjusted[b] HR (95% CI)	1.0	1.01 (0.75, 1.34)	0.98 (0.74, 1.31)	1.03 (0.78, 1.36)	1.07 (0.81, 1.42)	0.64
Pooled analysis						
Multivariate-adjusted[b] HR (95% CI)	1.0	1.05 (0.85, 1.28)	0.93 (0.76, 1.15)	0.95 (0.77, 1.16)	0.92 (0.68, 1.25)	0.52
β-Cryptoxanthin						
Women						
Median, mg/d	62	115	170	228	328	
Cases	61	103	108	119	91	
Age-adjusted[a] HR (95% CI)	1.0	1.40 (1.02, 1.93)	1.34 (0.98, 1.84)	1.41 (1.03, 1.92)	1.06 (0.76, 1.47)	0.75
Multivariate-adjusted[b] HR (95% CI)	1.0	1.33 (0.96, 1.83)	1.26 (0.91, 1.73)	1.29 (0.94, 1.77)	0.96 (0.69, 1.34)	0.35
Men						
Median, mg/d	66	126	188	256	384	
Cases	83	115	102	120	134	
Age-adjusted[a] HR (95% CI)	1.0	1.23 (0.93, 1.64)	1.05 (0.78, 1.41)	1.15 (0.87, 1.53)	1.26 (0.95, 1.67)	0.24
Multivariate-adjusted[b] HR (95% CI)	1.0	1.15 (0.86, 1.53)	0.94 (0.70, 1.26)	1.02 (0.76, 1.36)	1.06 (0.80, 1.41)	0.96
Pooled analysis						
Multivariate-adjusted[b] HR (95% CI)	1.0	1.22 (0.99, 1.51)	1.08 (0.81, 1.43)	1.14 (0.90, 1.43)	1.02 (0.82, 1.27)	0.56
Lutein						
Women						
Median, mg/d	1137	1903	2576	3383	5165	
Cases	97	99	102	97	87	
Age-adjusted[a] HR (95% CI)	1.0	0.91 (0.68, 1.20)	0.89 (0.67, 1.18)	0.84 (0.63, 1.12)	0.77 (0.58, 1.04)	0.09
Multivariate-adjusted[b] HR (95% CI)	1.0	0.89 (0.67, 1.19)	0.88 (0.66, 1.17)	0.85 (0.63, 1.13)	0.80 (0.59, 1.08)	0.18
Men						

Table 1.3 (Continued)

Median, mg/d	1263	2108	2866	3802	5838	
Cases	106	95	100	124	129	
Age-adjusted[a] HR (95% CI)	1.0	0.82 (0.62, 1.08)	0.82 (0.63, 1.09)	1.03 (0.79, 1.34)	1.07 (0.83, 1.39)	0.15
Multivariate-adjusted[b] HR (95% CI)	1.0	0.80 (0.60, 1.06)	0.81 (0.61, 1.07)	1.00 (0.77, 1.31)	1.06 (0.81, 1.38)	0.17
Pooled analysis						
Multivariate-adjusted[b] HR (95% CI)	1.0	0.85 (0.69, 1.03)	0.84 (0.69, 1.03)	0.93 (0.76, 1.13)	0.93 (0.70, 1.22)	0.95
Lycopene						
Women						
Median, mg/d	2783	4302	5553	7223	11001	
Cases	113	97	103	86	83	
Age-adjusted[a] HR (95% CI)	1.0	0.83 (0.63, 1.09)	0.91 (0.69, 1.18)	0.76 (0.57, 1.01)	0.85 (0.64, 1.14)	0.27
Multivariate-adjusted[b] HR (95% CI)	1.0	0.83 (0.63, 1.09)	0.92 (0.70, 1.21)	0.77 (0.58, 1.02)	0.90 (0.68, 1.21)	0.49
Men						
Median, mg/d	2592	4534	6125	8293	13426	
Cases	113	129	105	88	119	
Age-adjusted[a] HR (95% CI)	1.0	1.21 (0.93, 1.56)	0.99 (0.76, 1.30)	0.89 (0.67, 1.18)	1.26 (0.96, 1.63)	0.31
Multivariate-adjusted[b] HR (95% CI)	1.0	1.18 (0.91, 1.52)	0.95 (0.72, 1.24)	0.84 (0.63, 1.11)	1.22 (0.93, 1.58)	0.44
Pooled analysis						
Multivariate-adjusted[b] HR (95% CI)	1.0	0.99 (0.70, 1.40)	0.93 (0.77, 1.13)	0.80 (0.66, 0.98)	1.05 (0.79, 1.41)	0.80

Using cumulative average intake levels.

[a]Adjusted for age (years)

[b]Adjusted for age, pack years of smoking, coffee intake, BMI, physical activity, alcohol intake, and total energy intake

Chapter 2 Intake of Dairy Foods and Risk of Parkinson's Disease

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ABSTRACT

Importance: Prospective studies have suggested that dairy products may be associated with increased risk of Parkinson's disease (PD). However, it remains unclear which specific dairy products or associated nutrients are driving this association, and whether the association is present in women as well as men.

Objective: To examine the association between commonly consumed dairy products, as well as nutrients derived from dairy products, and risk of PD in men and women.

Design: Analyses were based on data from two large prospective cohort studies, the Health Professionals' Follow-up Study and the Nurses' Health Study, with a total of 24 and 26 years of follow-up, respectively.

Setting: Both US-based studies were conducted via mailed biennial questionnaires.

Participants: 48,672 men and 80,728 women who were free of PD at baseline and had non-missing baseline dietary information were included in analyses.

Exposures: Usual diet was assessed using food frequency questionnaires administered repeatedly over the follow-up period.

Main Outcome Measures: Incident cases of PD (n=1,035) were identified via questionnaires and subsequently confirmed by reviewing medical records. Multivariable hazard ratios were calculated in each cohort and pooled using meta-analysis.

Results: While total dairy intake was not associated with PD risk, intake of low-fat dairy foods was associated with PD risk. The pooled, multivariable-adjusted hazard ratio (HR) comparing people who consumed at least three servings of low-fat dairy per day to those who consumed none was 1.35 (95% CI, 1.01-1.79; *p* trend=0.04). This association appeared to be driven by an increased risk of PD associated with skim and low-fat milk (HR=1.60; 95% CI, 1.21-2.11; *p* trend=0.03). Results were similar in men and women.

Conclusions and Relevance: Consumption of certain dairy products, particularly low fat milk, is associated with an increased risk of PD in men and women.

INTRODUCTION

Evidence from prospective studies suggests a potential positive association between intake of dairy products and risk of Parkinson's disease (PD)[1 2,3 4]. Dairy products are widely consumed and as such could constitute an important modifiable risk factor for this disease. However, it remains unclear whether certain dairy foods or nutrients contained in dairy products are driving this association, and whether associations are present among women and men or only among men. Previous results from the Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS) suggested a positive association between dairy intake and PD risk among men[1]. Here we present data from the NHS and HPFS cohorts from up to an additional 12 years of follow-up with a total of 1,035 incident cases of PD to further investigate potential associations between dairy foods, and nutrients derived from dairy products, and risk of PD.

METHODS

Study population

The Nurses' Health Study cohort began in 1976 when 121,700 female registered nurses aged 30 to 55 years responded to a mailed questionnaire, answering questions regarding their medical histories and health-related behaviors. Similarly, the Health Professionals Follow-up Study was established when 51,529 male health professionals aged 40 to 75 years responded to a similar questionnaire. Members of both cohorts have been sent follow-up questionnaires every two years to update information on health-related exposures and newly diagnosed diseases. For the present analysis, the baseline population was composed of the 81,757 nurses and 49,934 health professionals who completed baseline food frequency questionnaires regarding usual dietary intake. We excluded individuals who reported implausible total energy intake at baseline (<660 or >3500 kcal/day for women and <800 or >4200

kcal/day for men), had a previous diagnosis of PD, or had missing baseline dietary information. A total of 48,672 men and 80,728 women were thus included in the analysis. This study was approved by the Human Research Committees at the Brigham and Women's Hospital and the Harvard T. H. Chan School of Public Health.

Dietary assessment and other covariates

Usual diet was assessed through food frequency questionnaires. Participants were asked how often over the past year they consumed a commonly used portion of each food. Nine possible responses were provided, ranging from "never" to "six or more times per day". Nutrient intakes were calculated by multiplying the frequency response by the nutrient content of the specified portion size, based on data from the US Department of Agriculture and manufacturers. Participants were also asked about supplemental vitamin use. Diet was assessed in the NHS in 1984 (baseline), 1986, and every four years thereafter, and in HPFS in 1986 (baseline) and every four years thereafter. Intakes of foods and nutrients assessed in this way have been validated previously against diet records among subsets of participants in both the NHS and HPFS cohorts[5-8]. Correlations between intakes of dairy foods measured by food frequency questionnaire and by diet records were 0.52-0.88 in HPFS[8] and 0.57-0.94 in NHS[7]. Data on other covariates of interest were also collected via self-report questionnaires for both cohorts.

Ascertainment of PD cases

Cases of PD were identified using biennial self-report questionnaires. Prior to 2003, when individuals indicated a diagnosis of PD, we contacted their treating neurologists or internists who were asked to either confirm the diagnosis or send copies of the patients' medical records. Cases were confirmed if the physician considered the PD diagnosis definite or probable, the medical record included a final diagnosis of PD by a neurologist, or the medical record indicated the presence of at least two of

the three cardinal signs of PD (resting tremor, rigidity, bradykinesia) in the absence of evidence for other diagnoses. Since 2003, the above procedure was used with the exception that medical records were requested for all self-reported cases and were reviewed by a neurologist specializing in movement disorders. In the event that the determination of the movement disorders specialist differed from that of the neurologist, the decision of the movement disorder specialist was used. Our analyses included only confirmed cases.

Statistical analysis

Participants contributed person-time from the age in months at the date of returning the baseline food frequency questionnaire until the age in months at the date of PD diagnosis, death, last completed questionnaire, or end of follow-up (June 2010 for NHS and January 2010 for HPFS), whichever occurred first. Analyses were stratified by age in months at start of follow-up and calendar year of current questionnaire cycle. PD incidence was related to cumulative updated average intake from all available questionnaires up to the start of each two-year follow-up period, categorized by cohort-specific quintile of intake[9] for nutrient analyses and into four-level variables for individual dairy foods. We adjusted for the number of missing food frequency questionnaires using indicator variables. In each analysis, the lowest intake category was used as the reference group. Age-adjusted and multivariate-adjusted hazard ratios were estimated for each exposure using Cox proportional hazards models. Separate models were fit for each exposure of interest: total dairy; dairy protein; calcium from all sources, from foods, and from dairy sources; vitamin D from all sources, from foods, and from dairy sources; lactose; and individual dairy products, including skim or low-fat milk, whole milk, cream, cream cheese, cottage cheese, other cheese, ice cream, yogurt, sherbet or frozen yogurt, butter, and margarine. Nutrients were adjusted for total energy using the residual method so that they would be uncorrelated with total energy intake[10]. We also investigated high fat dairy, including whole fat milk,

cream, ice cream, sour cream, butter, cream cheese and other cheese, and low fat dairy, including skim and low fat milk, sherbet/frozen yogurt, yogurt, cottage cheese and low-fat cheese. Primary multivariate models were adjusted for pack years of smoking (never smoker, 1 to <5, 5 to <10, 10 to <15, ≥15), coffee intake (non, <1, 1-3, 4-5, or ≥6 cups/day), body mass index (BMI, <21, 21 to <25, 25 to <30, 30 to <35, or ≥35 kg/m2), physical activity (in quintiles), alcohol intake (none, 0.1 to 4.9, 5.0 to 9.9, 10.0 to 14.9, or ≥15 g/day for women and none, 0.1 to 9.9, 10.0 to 19.9, 20.0 to 29.9, or ≥30 g/day for men), and total energy intake (in quintiles). The robustness of the results was tested by further adjustment for dietary patterns[11] and flavonoids intake[12]. We conducted a lagged analysis excluding the first four years of follow-up in each cohort to address the possibility that participants could be experiencing PD symptoms at the time of questionnaire completion.

We also conducted a meta-analysis to combine our study with previously published prospective studies on milk intake and PD risk. We identified relevant studies through a PubMed search using keywords (dairy or milk) and (Parkinson or Parkinson's disease) for all published studies in English by January 27, 2016. We identified five prospective studies of milk intake and PD risk[1,2,3,13,4] (Appendix Table 2.4). However, since the previous HPFS/NHS study[1] overlaps with the first 12 years of follow-up of the current study, it was not included in the meta-analysis. We also excluded one study[13] that reported effect estimates for a one standard deviation increase in milk intake rather than comparing extreme intake categories. We used the I^2 statistic to assess heterogeneity across studies and used random effects models to calculate the pooled relative risk. All statistical analyses were conducted using SAS (SAS Institute, Cary, NC) with the exception of the meta-analysis, which was conducted using Stata (Version 11.2).

RESULTS

We identified 554 incident PD cases in men and 481 in women over the follow-up period. Selected baseline characteristics of cohort participants according to their dairy intake are shown in Table 2.1. In the main analysis, the association between total dairy and PD risk was not significant (pooled multivariable-adjusted HR comparing those who eat at least three servings of dairy foods per day to those who eat less than one serving per day = 1.17; 95% CI 0.92-1.49; *p* trend=0.18) (Table 2.2). However, when looking specifically at low fat dairy foods, we found an elevated risk of PD for individuals with high intake (pooled HR comparing extreme intake categories= 1.35; 95% CI 1.01-1.79; *p* trend=0.04). For high fat dairy foods, the associations tended to be in the opposite direction. Although the HR comparing the highest category of intake to the lowest was not significant (HR=0.82, 95% CI 0.61-1.10), we found a significant linear trend for decreased risk associated with greater intake of high fat dairy (*p* trend = 0.03). Results were similar when we categorized dairy intake by quintiles rather than categories (Figure 2.1). The elevated risk associated with low fat dairy foods appeared to be driven by an association between PD and skim or low fat milk (Table 2.3). After conducting a four-year lag analysis, the association between skim or low fat milk and PD risk was strengthened (the HR comparing those who drink more than one serving per day to never drinker increased from 1.60 [95% CI 1.21-2.11] in the main analysis to 1.67 [95% CI 1.23-2.25] in the lagged analysis). The pooled results suggest an increased PD risk in all categories of low-fat milk consumption as compared to no consumption. Results were similar in men and women. In addition to low fat milk, intake of sherbet/frozen yogurt was also associated with an increased risk of PD. In contrast, while whole milk did not appear to be associated with PD risk in men, it was associated with a decreased risk of PD among women in both the main (Table 2.3) and the four-year lag analysis (HR=0.39, 95% CI 0.16-0.96, *p* trend=0.02). We conducted further analyses including low fat and whole fat milk in the same model, and found similar results. For low fat milk, the pooled HR comparing extreme categories was 1.58 (95% CI 1.19-2.10, *p* trend 0.03), and similar

to the main analysis we found an inverse association for whole fat milk only among women (HR=0.44, 95% CI 0.16-1.20, *p* trend 0.04).

Table 2.1 Age-adjusted characteristics of the study population at baseline by category of dairy intake

	Total dairy intake		Low fat dairy intake		High fat dairy intake	
	<1 per day	≥ 3 per day	<1 per day	≥ 3 per day	<1 per day	≥ 3 per day
Nurses' Health Study, 1984-2010	n=18250	n=16195	n=48877	n=3433	n=37376	n=10185
Age, years*	50.5(7.0)	51.24(7.21)	50.58(7.09)	52.6(7.1)	51.3(7.2)	50.5(7.1)
Body mass index, kg/m	25.7(4.9)	25.90(5.03)	25.72(4.91)	26.4(5.2)	25.9(4.9)	25.6(5.1)
Current smoker, %	30	23	28	17	23	31
Past smoker, %	30	32	30	35	33	29
Caucasian, %	96	98	97	99	97	99
Activity, met-h/week	12.7(18.6)	15.2(24.0)	13.2(20.3)	18.6(30.5)	14.4(20.6)	13.4(22.2)
Coffee, servings/day	1.8(1.8)	1.8(1.8)	1.9(1.8)	1.6(1.7)	1.6(1.7)	2.2(1.8)
Alcohol, g/day	7.9 (12.8)	6.0(10.2)	7.6(12.1)	4.6(8.6)	6.3(10.9)	7.8(12.1)
Total energy intake, kcal/day	1434(448)	2104(515)	1662(522)	2157(503)	1568(468)	2105(542)
Health Professionals Follow-up Study, 1986-2010	n=12313	n=9707	n=29301	n=2434	n=26897	n=4467
Age, years*	54.5(9.5)	55.1(10.0)	54.5(9.7)	56.0(10.0)	55.1(9.8)	55.1(9.9)
Body mass index, kg/m	25.4(3.4)	25.6(3.4)	25.6(3.4)	25.6(3.5)	25.4(3.3)	25.6(3.4)
Current smoker, %	11	11	11	7	9	17
Past smoker, %	46	39	45	37	45	39
Caucasian, %	92	98	95	98	95	97

Table 2.1 (Continued)

	Total dairy intake		Low fat dairy intake		High fat dairy intake	
	<1 per day	≥ 3 per day	<1 per day	≥ 3 per day	<1 per day	≥ 3 per day
Activity, met-h/week	17.3(24.6)	19.4(27.2)	17.7(24.9)	21.2(27.7)	19.2(26.2)	17.3(23.9)
Coffee, servings/day	1.3(1.6)	1.5(1.7)	1.4(1.6)	1.1(1.6)	1.2(1.5)	1.9(1.8)
Alcohol, g/day	12.1(16.5)	10.4(15.1)	12.3(16.2)	8.3(13.6)	10.7(14.9)	13.2(17.5)
Total energy intake, kcal/day	1669(526)	2412(626)	1885(604)	2520(620)	1815(551)	2476(662)

Values are means(SD) or percentages and are standardized to the age distribution of the study population

[a] Metabolic equivalents from recreational and leisure-time activities

[*] Value is not age-adjusted

Table 2.2 Relative risk of PD by intake of total, high fat, and low fat dairy foods

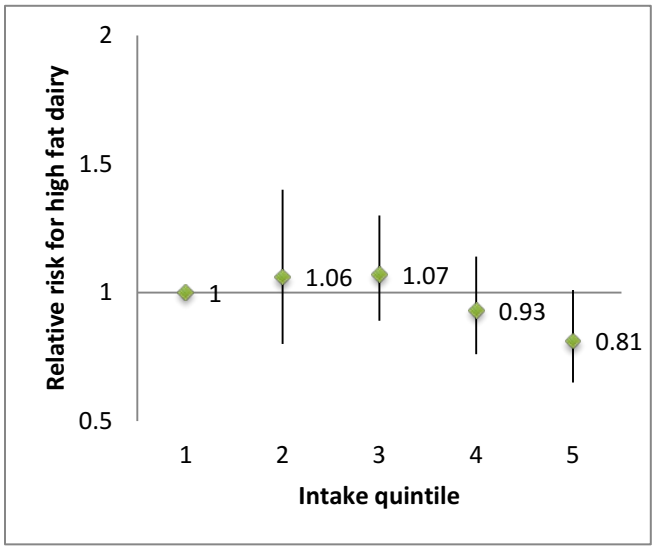
Dairy category	Categories (servings/week or day)				P_{trend}
Total dairy	< 1/day	< 2/day	< 3/day	>= 3/day	
Women					
Number of cases	52	183	158	88	
Person-years	282961	730242	503552	353554	
Relative risk (95% CI)					
Age-adjusted	1.0	1.21 (0.89, 1.65)	1.43 (1.04, 1.96)	1.22 (0.86, 1.72)	p=0.23
Multivariate*	1.0	1.15 (0.84, 1.59)	1.30 (0.93, 1.82)	1.09 (0.74, 1.59)	p=0.77
Men					
Number of cases	100	214	134	106	
Person-years	202275	370305	199172	165255	
Relative risk (95% CI)					
Age-adjusted	1.0	1.09 (0.86, 1.38)	1.20 (0.92, 1.56)	1.15 (0.87, 1.51)	p=0.26
Multivariate*	1.0	1.10 (0.86, 1.41)	1.25 (0.95, 1.66)	1.24 (0.91, 1.68)	P=0.13
Pooled analysis					
Multivariate*	1.0	1.12 (0.92, 1.36)	1.27 (1.03, 1.58)	1.17 (0.92, 1.49)	p=0.18
Low fat dairy	< 1/day	< 2/day	< 3/day	>= 3/day	
Women					
Number of cases	218	184	61	18	
Person-years	1033694	568816	208916	58132	
Relative risk (95% CI)					
Age-adjusted	1.0	1.36 (1.12, 1.66)	1.25 (0.94, 1.66)	1.33 (0.82, 2.16)	p=0.02
Multivariate*	1.0	1.30 (1.06, 1.59)	1.15 (0.86, 1.54)	1.20 (0.73, 1.98)	p=0.12
Men					
Number of cases	262	179	66	42	
Person-years	509371	264782	110604	45917	
Relative risk (95% CI)					

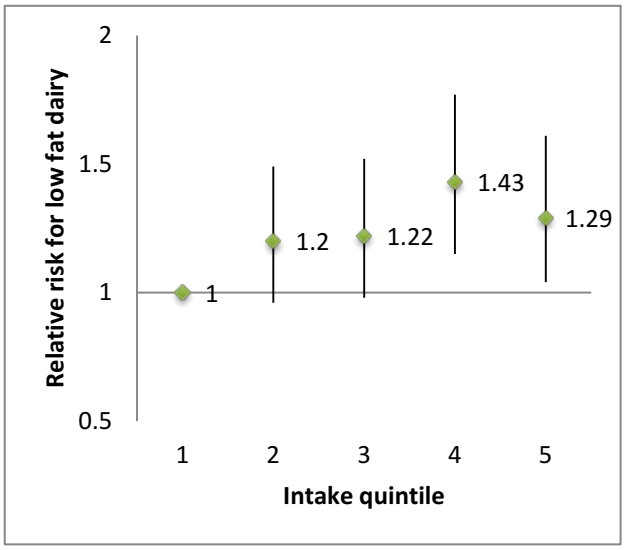
Table 2.2 (Continued)

Age-adjusted	1.0	1.19 (0.98, 1.44)	1.01 (0.77, 1.33)	1.48 (1.06, 2.06)	p=0.07
Multivariate*	1.0	1.15 (0.94, 1.40)	0.98 (0.74, 1.30)	1.42 (1.00, 2.02)	P=0.16
Pooled analysis					
Multivariate*	1.0	1.22 (1.06, 1.41)	1.06 (0.86, 1.30)	1.35 (1.01, 1.79)	p=0.04
High fat dairy	< 1/day	< 2/day	< 3/day	>= 3/day	
Women					
Number of cases	282	144	32	23	
Person-years	984925	563753	184798	136528	
Relative risk (95% CI)					
Age-adjusted	1.0	1.01 (0.83, 1.24)	0.74 (0.51, 1.07)	0.85 (0.55, 1.30)	p=0.19
Multivariate*	1.0	0.99 (0.80, 1.22)	0.73 (0.50, 1.06)	0.86 (0.55, 1.34)	p=0.21
Men					
Number of cases	348	137	37	31	
Person-years	520305	268215	80406	65015	
Relative risk (95% CI)					
Age-adjusted	1.0	0.81 (0.66, 0.99)	0.71 (0.50, 1.00)	0.72 (0.53, 1.05)	p=0.01
Multivariate*	1.0	0.83 (0.68, 1.03)	0.75 (0.53, 1.07)	0.79 (0.53, 1.16)	p=0.07
Pooled analysis					
Multivariate*	1.0	0.91 (0.77, 1.08)	0.74 (0.57, 0.96)	0.82 (0.61, 1.10)	p=0.03

Using cumulative average intake levels.

*Adjusted for age, pack years of smoking, coffee intake, BMI, physical activity, alcohol intake, and total energy intake





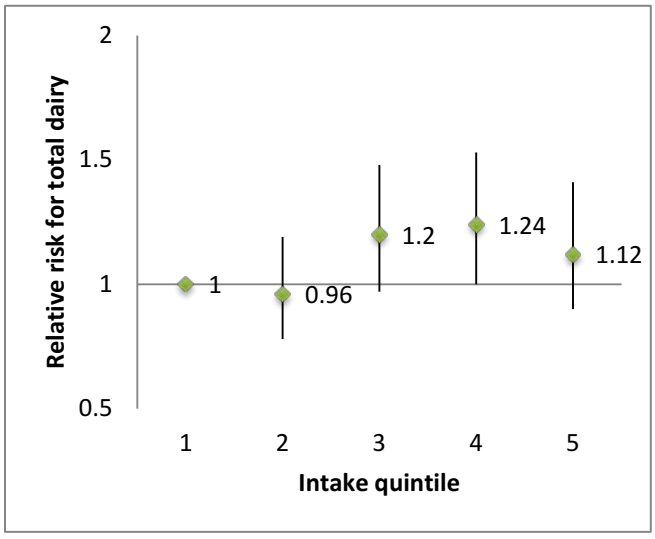


Figure 2.1 Associations of high fat, low fat, and total dairy with risk of PD according to intake quintile, using cumulative average intake levels and adjusted for pack years of smoking, physical activity, coffee intake, body mass index, alcohol intake, and total energy intake.

Table 2.3 Relative risk of PD by intake of individual dairy products

Dairy product	Categories (servings/week or day)				P_{trend}
Total milk	Never	≤ 1/week	≤ 1/day	> 1/day	
Women					
Number of cases	10	46	212	213	
Person-years	97328	227635	860467	683558	
Relative risk (95% CI)					
Age-adjusted	1.0	1.64 (0.82, 3.26)	1.53 (0.81, 2.90)	1.93 (1.02, 3.66)	p<0.01
Multivariate*	1.0	1.56 (0.78, 3.11)	1.38 (0.73, 2.63)	1.65 (0.86, 3.16)	p=0.08
Men					
Number of cases	22	55	258	217	
Person-years	76725	114743	410694	330660	
Relative risk (95% CI)					
Age-adjusted	1.0	1.60 (0.97, 2.64)	1.74 (1.12, 2.70)	1.80 (1.15, 2.80)	p=0.15
Multivariate*	1.0	1.58 (0.96, 2.60)	1.65 (1.06, 2.57)	1.73 (1.10, 2.70)	p=0.20
Pooled analysis					
Multivariate*	1.0	1.57 (1.05, 2.35)	1.56 (1.08, 2.25)	1.70 (1.18, 2.46)	p=0.03
Skim milk or low-fat milk	Never	≤ 1/week	≤ 1/day	> 1/day	
Women					
Number of cases	23	49	209	194	
Person-years	224345	233765	809951	574826	
Relative risk (95% CI)					
Age-adjusted	1.0	1.49 (0.90, 2.47)	1.48 (0.95, 2.29)	1.94 (1.25, 3.01)	p<0.01
Multivariate*	1.0	1.43 (0.86, 2.36)	1.35 (0.86, 2.10)	1.68 (1.07, 2.63)	p=0.01
Men					
Number of cases	40	61	238	189	
Person-years	127303	111935	378754	289438	
Relative risk (95% CI)					

Table 2.3 (Continued)

Age-adjusted	1.0	1.64 (1.10, 2.46)	1.61 (1.14, 2.26)	1.68 (1.19, 2.37)	p=0.13
Multivariate*	1.0	1.60 (1.07, 2.41)	1.48 (1.05, 2.09)	1.56 (1.09, 2.21)	p=0.26
Pooled analysis					
Multivariate*	1.0	1.53 (1.12, 2.10)	1.43 (1.09, 1.88)	1.60 (1.21, 2.11)	p=0.03
Whole milk	Never	≤ 1/week	≤ 1/day	> 1/day	
Women					
Number of cases	277	123	63	4	
Person-years	1040657	446988	277923	60770	
Relative risk (95% CI)					
Age-adjusted	1.0	0.96 (0.78, 1.19)	0.83 (0.63, 1.09)	0.36 (0.13, 0.97)	p=0.01
Multivariate*	1.0	0.95 (0.77, 1.18)	0.82 (0.62, 1.08)	0.36 (0.13, 0.98)	p=0.01
Men					
Number of cases	330	80	65	15	
Person-years	580934	156055	90687	28335	
Relative risk (95% CI)					
Age-adjusted	1.0	0.89 (0.70, 1.14)	1.14 (0.87, 1.49)	0.89 (0.52, 1.51)	p=0.93
Multivariate*	1.0	0.90 (0.70, 1.16)	1.19 (0.90, 1.56)	0.99 (0.58, 1.69)	p=0.56
Pooled analysis					
Multivariate*	1.0	0.93 (0.79, 1.09)	0.99 (0.69, 1.42)	0.66 (0.25, 1.73)	p=0.50
Cream	Never	1-3/month	≤ 1/week	> 1/week	
Women					
Number of cases	189	165	38	75	
Person-years	830505	575240	103262	315619	
Relative risk (95% CI)					
Age-adjusted	1.0	0.96 (0.77, 1.19)	1.12 (0.78, 1.60)	0.86 (0.65, 1.13)	p=0.30
Multivariate*	1.0	0.91 (0.73, 1.13)	1.06 (0.74, 1.52)	0.84 (0.63, 1.11)	p=0.31
Men					
Number of cases	279	104	20	74	

Table 2.3 (Continued)

Person-years	497507	184510	36940	132580	
Relative risk (95% CI)					
Age-adjusted	1.0	0.95 (0.75, 1.21)	0.90 (0.57, 1.43)	0.91 (0.70, 1.18)	p=0.45
Multivariate*	1.0	0.90 (0.71, 1.14)	0.84 (0.53, 1.34)	0.91 (0.70, 1.19)	p=0.55
Pooled analysis					
Multivariate*	1.0	0.90 (0.77, 1.06)	0.97 (0.73, 1.29)	0.87 (0.72, 1.06)	p=0.26
Cream cheese	Never	1-3/month	≤ 1/week	> 1/week	
Women					
Number of cases	135	239	58	41	
Person-years	591685	880087	212370	154707	
Relative risk (95% CI)					
Age-adjusted	1.0	0.99 (0.79, 1.22)	0.93 (0.68, 1.28)	0.88 (0.62, 1.25)	p=0.45
Multivariate*	1.0	0.96 (0.77, 1.19)	0.90 (0.65, 1.24)	0.86 (0.60, 1.23)	p=0.37
Men					
Number of cases	189	200	56	54	
Person-years	384873	335079	75005	66183	
Relative risk (95% CI)					
Age-adjusted	1.0	1.10 (0.90, 1.35)	1.38 (1.02, 1.87)	1.22 (0.90, 1.66)	p=0.10
Multivariate*	1.0	1.08 (0.88, 1.33)	1.37 (1.01, 1.86)	1.21 (0.89, 1.66)	p=0.10
Pooled analysis					
Multivariate*	1.0	1.02 (0.88, 1.18)	1.11 (0.74, 1.68)	1.03 (0.73, 1.45)	p=0.81
Cottage cheese	Never	1-3/month	≤ 1/week	> 1/week	
Women					
Number of cases	56	186	100	135	
Person-years	276230	712403	355171	508088	
Relative risk (95% CI)					
Age-adjusted	1.0	1.07 (0.79, 1.45)	1.04 (0.74, 1.44)	0.90 (0.66, 1.24)	p=0.24
Multivariate*	1.0	1.05 (0.77, 1.42)	0.99 (0.71, 1.39)	0.86 (0.62, 1.18)	p=0.16

Table 2.3 (Continued)

Men					
Number of cases	113	184	84	128	
Person-years	233647	337692	143091	169964	
Relative risk (95% CI)					
Age-adjusted	1.0	0.98 (0.77, 1.25)	0.99 (0.75, 1.32)	1.07 (0.83, 1.38)	p=0.63
Multivariate*	1.0	0.97 (0.76, 1.23)	1.00 (0.75, 1.33)	1.08 (0.83, 1.41)	p=0.53
Pooled analysis					
Multivariate*	1.0	1.00 (0.83, 1.21)	1.00 (0.80, 1.24)	0.98 (0.78, 1.23)	p=0.70
Other cheese	≤3/month	≤ 1/week	≤ 2-4/week	≥ 5-6/week	
Women					
Number of cases	39	50	239	152	
Person-years	169174	262278	877076	554417	
Relative risk (95% CI)					
Age-adjusted	1.0	0.79 (0.52, 1.21)	1.00 (0.71, 1.41)	1.12 (0.78, 1.60)	p=0.07
Multivariate*	1.0	0.79 (0.52, 1.21)	0.97 (0.68, 1.37)	1.08 (0.75, 1.57)	p=0.11
Men					
Number of cases	88	67	266	119	
Person-years	154051	141258	403607	216486	
Relative risk (95% CI)					
Age-adjusted	1.0	0.90 (0.65, 1.24)	1.17 (0.91, 1.49)	0.99 (0.75, 1.31)	p=0.76
Multivariate*	1.0	0.95 (0.69, 1.31)	1.25 (0.97, 1.61)	1.12 (0.83, 1.50)	p=0.29
Pooled analysis					
Multivariate*	1.0	0.86 (0.69, 1.15)	1.13 (0.88, 1.45)	1.10 (0.87, 1.39)	p=0.07
Ice cream	Never	1-3/month	≤ 1/week	> 1/week	
Women					
Number of cases	31	173	97	175	
Person-years	166901	721964	372969	588853	
Relative risk (95% CI)					

Table 2.3 (Continued)

Age-adjusted	1.0	1.07 (0.73, 1.57)	1.13 (0.75, 1.70)	1.22 (0.83, 1.79)	p=0.19
Multivariate*	1.0	0.98 (0.66, 1.44)	0.98 (0.64, 1.48)	1.01 (0.68, 1.50)	p=0.84
Men					
Number of cases	74	195	98	164	
Person-years	116203	351656	173221	262304	
Relative risk (95% CI)					
Age-adjusted	1.0	0.84 (0.64, 1.11)	0.86 (0.63, 1.17)	0.83 (0.63, 1.10)	p=0.95
Multivariate*	1.0	0.81 (0.61, 1.06)	0.82 (0.60, 1.12)	0.78 (0.58, 1.04)	p=0.72
Pooled analysis					
Multivariate*	1.0	0.86 (0.69, 1.08)	0.87 (0.68, 1.12)	0.86 (0.67, 1.09)	p=0.90
Yogurt	Never	1-3/month	≤ 1/week	> 1/week	
Women					
Number of cases	115	131	71	152	
Person-years	610397	485360	236255	508369	
Relative risk (95% CI)					
Age-adjusted	1.0	1.21 (0.93, 1.55)	1.28 (0.94, 1.73)	1.24 (0.96, 1.59)	p=0.20
Multivariate*	1.0	1.17 (0.90, 1.51)	1.23 (0.91, 1.67)	1.17 (0.90, 1.51)	p=0.42
Men					
Number of cases	222	116	51	101	
Person-years	402321	225912	85585	150070	
Relative risk (95% CI)					
Age-adjusted	1.0	0.94 (0.74, 1.18)	1.10 (0.81, 1.50)	1.17 (0.92, 1.49)	p=0.14
Multivariate*	1.0	0.87 (0.69, 1.10)	1.02 (0.74, 1.39)	1.07 (0.84, 1.37)	p=0.39
Pooled analysis					
Multivariate*	1.0	1.00 (0.75, 1.33)	1.12 (0.90, 1.40)	1.12 (0.93, 1.33)	p=0.24
Sherbet	Never	1-3/month	≤ 1/week	> 1/week	
Women					
Number of cases	91	170	87	120	

Table 2.3 (Continued)

Person-years	573425	707925	230937	316467	
Relative risk (95% CI)					
Age-adjusted	1.0	1.02 (0.78, 1.33)	1.37 (1.00, 1.86)	1.32 (0.99, 1.75)	p=0.01
Multivariate*	1.0	0.94 (0.72, 1.23)	1.23 (0.90, 1.68)	1.16 (0.86, 1.55)	p=0.08
Men					
Number of cases	124	181	54	132	
Person-years	285639	318686	99397	146037	
Relative risk (95% CI)					
Age-adjusted	1.0	1.12 (0.89, 1.42)	1.01 (0.72, 1.40)	1.42 (1.10, 1.83)	p<0.01
Multivariate*	1.0	1.03 (0.81, 1.32)	0.93 (0.66, 1.30)	1.28 (0.98, 1.66)	p=0.03
Pooled analysis					
Multivariate*	1.0	0.99 (0.83, 1.18)	1.07 (0.81, 1.40)	1.22 (1.01, 1.48)	p<0.01
Butter	Never	≤ 1/week	≤ 1/day	> 1/day	
Women					
Number of cases	139	146	152	36	
Person-years	635904	481426	575171	161242	
Relative risk (95% CI)					
Age-adjusted	1.0	1.10 (0.87, 1.40)	1.02 (0.81, 1.30)	0.94 (0.65, 1.36)	p=0.63
Multivariate*	1.0	1.09 (0.85, 1.38)	1.04 (0.82, 1.32)	0.95 (0.65, 1.39)	p=0.74
Men					
Number of cases	203	172	133	21	
Person-years	319230	281557	247178	48741	
Relative risk (95% CI)					
Age-adjusted	1.0	0.99 (0.80, 1.22)	0.86 (0.69, 1.08)	0.66 (0.42, 1.04)	p=0.04
Multivariate*	1.0	0.95 (0.77, 1.17)	0.86 (0.69, 1.09)	0.69 (0.44, 1.10)	p=0.09
Pooled analysis					
Multivariate*	1.0	1.01 (0.86, 1.18)	0.94 (0.79, 1.13)	0.83 (0.61, 1.14)	p=0.18
Margarine	Never	≤ 1/week	≤ 1/day	> 1/day	

Table 2.3 (Continued)

Women					
Number of cases	25	46	266	142	
Person-years	176573	201396	950404	527105	
Relative risk (95% CI)					
Age-adjusted	1.0	1.31 (0.80, 2.14)	1.57 (1.04, 2.37)	1.44 (0.94, 2.21)	p=0.56
Multivariate*	1.0	1.24 (0.76, 2.03)	1.44 (0.95, 2.18)	1.26 (0.82, 1.94)	p=0.89
Men					
Number of cases	79	80	266	106	
Person-years	139410	149353	469231	153301	
Relative risk (95% CI)					
Age-adjusted	1.0	0.99 (0.72, 1.35)	0.95 (0.74, 1.23)	0.95 (0.70, 1.27)	p=0.95
Multivariate*	1.0	0.94 (0.68, 1.29)	0.94 (0.73, 1.22)	0.95 (0.70, 1.29)	p=0.84
Pooled analysis					
Multivariate*	1.0	1.02 (0.78, 1.33)	1.13 (0.75, 1.71)	1.05 (0.81, 1.36)	p=0.96

Using cumulative average intake levels.

*Adjusted for age, pack years of smoking, coffee intake, BMI, physical activity, alcohol intake, and total energy intake

We conducted further analyses of specific nutrients found in dairy products, including protein, calcium, vitamin D, and lactose. Neither total intakes nor intakes from dairy only were significantly associated with PD risk (Appendix Table 2.5). Results of lagged analyses were overall similar to those of the main analyses. We conducted further sensitivity analyses by restricting our case definition to definite PD, by analyzing only non-smokers, and by censoring participants at age 85 because of the increasing difficulty of diagnosing PD at older ages. Again results were similar to those of the main analyses. We also evaluated potential effect modification for the effect of low fat dairy by running our analyses stratified by age (above vs. below 65 years old), smoking (ever vs. never smokers), and coffee (drinkers vs. non-drinkers). No significant interactions were observed.

Finally, we pooled the present study with 3 previously published studies [2,3,4] with a total of 1636 PD cases. The pooled RR for total milk intake was 1.80 (95% CI 1.44-2.25) (Figure 2.2).

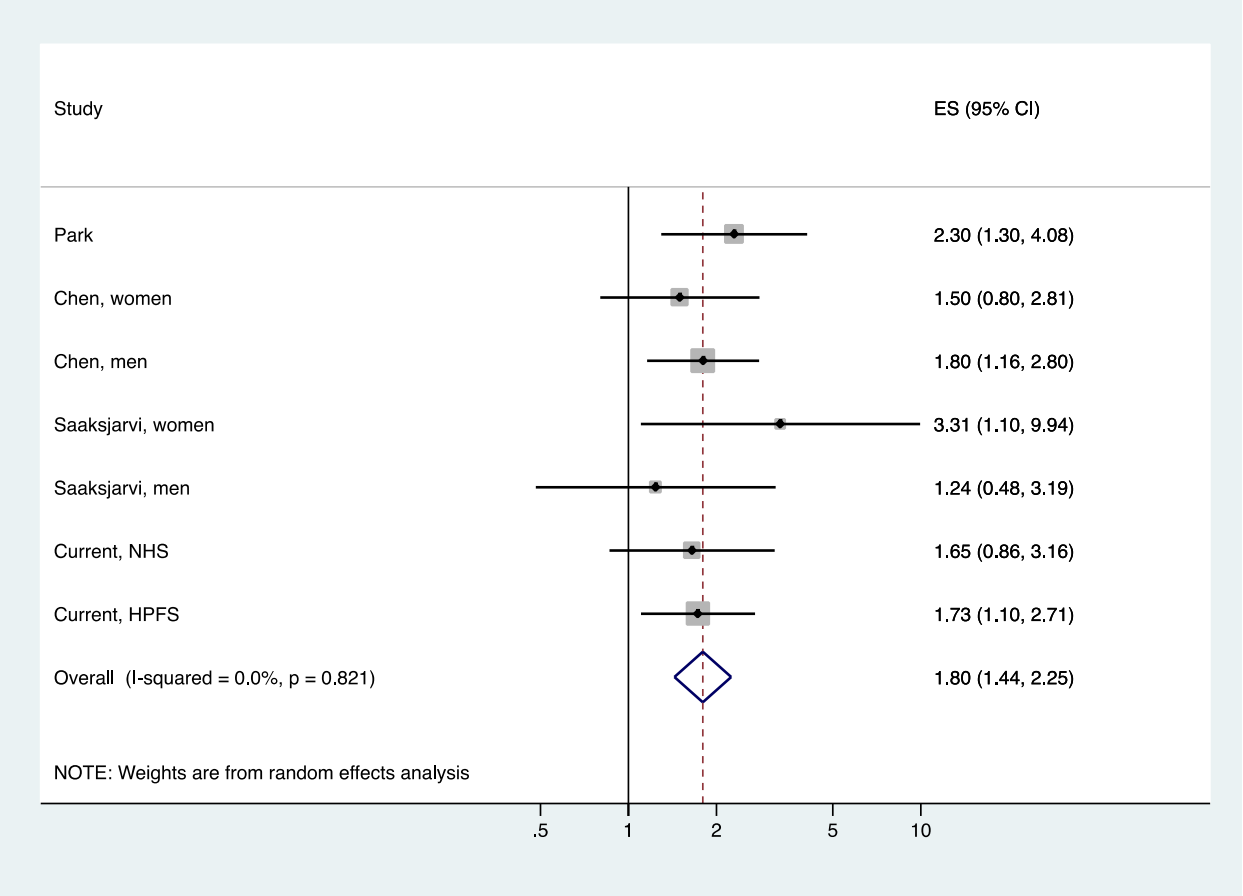


Figure 2.2 Forest plot for milk intake and risk of Parkinson's disease. P-value for overall pooled estimate < 0.00001.

DISCUSSION

In this large prospective study with over 1,000 incident cases of PD, we found evidence for a positive association between PD risk and certain dairy products. Low fat dairy products were associated with an increased risk of PD, and skim or low fat milk and frozen yogurt appeared to contribute to this association.

The strengths of our study include the prospective design, which reduces the potential for reverse causation and recall bias that can affect retrospective studies of diet and disease. Both cohorts also had high follow-up rates and employed validated dietary questionnaires, and exposure assessments

were conducted repeatedly over the follow-up period. Our study is also the largest analysis of dairy and PD to date, with over 1000 incident PD cases.

Our study also has some weaknesses. Although previous validation studies suggest that our food frequency questionnaire captures intake levels in these cohorts reasonably well, some degree of misclassification of diet is inevitable. However, exposure misclassification is unlikely to explain the positive associations since we would expect this bias to attenuate associations toward the null due to the prospective nature of our study. It is also possible that early symptoms of PD affected dietary behaviors or questionnaire responses; however, the results of our lag analysis suggest that any reverse causation was modest.

Our results largely agree with previous epidemiologic investigations of the association between dairy products and PD. This association in HPFS and NHS was first investigated by Chen and colleagues [1], who identified nearly 400 cases over 12 to 18 years of follow-up and reported a positive association between PD risk and dairy intake among men, but not among women. An increased risk of PD associated with milk consumption was also observed in other cohorts, including the Cancer Prevention Study[3], the Honolulu Heart Program cohort [2], and the Finnish Mobile Clinic Survey [4], although the latter reported an association only among women. Of note, only 45 male cases were included in the analysis, so low power could have affected the results. In addition, most of the milk consumed in the Finnish cohort was whole milk, whereas in our study population skim and low fat milk were more widely used.

One mechanism that has been proposed to explain the apparent association between certain dairy products and increased risk of PD is through the antiuricemic effect of dairy proteins. A substantial body of evidence suggests that uric acid may be protective against PD[14-16]. Milk proteins (casein and lactalbumin) have been shown to reduce serum uric acid levels in healthy subjects[17], and consumption of low fat, but not high fat, dairy has been associated with a reduced risk of gout among participants in

the HPFS[18]. The lack of association with full-fat dairy products could be due to counteracting effect of saturated fats[19]. An inverse association between total milk or yogurt consumption and serum urate level was also found in NHANES, but data for full-fat or low fat dairy were not reported[20]. According to the results of Choi et al[20], consumption of more than two servings of dairy per day was associated with -0.19 mg/dL lower serum uric acid (95% CI -0.30, -0.09), while greater than one serving of milk per day was associated with -0.25 mg/dL lower serum uric acid (95% CI -0.40, -0.09). While this might contribute to the observed association between milk and PD risk, it may be too modest of a change in uric acid level to completely explain our results. Another possible mechanism is through possible contaminants found in dairy products, such as pesticides. Recently an inverse association was reported between milk intake and neuronal density in the substantia nigra among non-smokers in the Honolulu-Asia Aging Study [21]. The same study also found an association between detectable heptachlor epoxide (which contaminated milk in Hawaii in the early 1980s) in the brain and milk intake among non-smokers. However, in our study we found similar associations between milk and PD among both smokers and non-smokers, and the consistent finding of an increasing risk of PD with increasing milk intake across multiple studies seems more consistent with a general mechanisms rather than specific contaminants.

In conclusion, our results provide further evidence of an increased risk of PD associated with consumption of certain dairy products in men and women, and in particular with low fat milk. Further research is needed to elucidate the mechanisms involved in this association.

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APPENDIX

Table 2.4 Prospective studies of milk and PD risk

First Author, year	Study design	Population	Exposure	Outcome	Results
Chen, 2002	Cohort	88,563 women from the Nurses' Health Study and 47,331 men from the Health Professionals Follow-up Study	Total dairy, low-fat dairy, high-fat dairy, individual dairy foods	184 incident PD cases in NHS and 210 incident PD cases in HPFS	RR comparing extreme quintiles of total dairy intake =1.8 (95% CI 1.2-2.8, *p* trend=0.004) in men and 1.1 (95% CI 0.7-1.7, *p* trend=0.9) in women, adjusting for age, lengths of follow-up, smoking, energy intake, caffeine intake, body mass index, alcohol consumption, and physical activity
Park, 2005	Cohort	7,504 men from the Honolulu –Asia Aging Study	Milk intake	128 incident PD cases	RR comparing highest intake group (>16 oz/day) to those who consume no milk was 2.3 (95% CI 1.3-4.1) adjusting for age, coffee intake, smoking pack-years, physical activity, tricep skinfold thickness, total kilocalories and fat intake, and years working on a plantation
Chen, 2007	Cohort	73,175 women and 57,689 men from the Cancer Prevention Study II Nutrition Cohort	Total dairy, individual dairy foods	138 incident PD cases in women and 250 incident PD cases in men	RR comparing extreme quintile of total dairy intake was 1.6 (95% CI 1.0-2.5, *p* trend=0.04) in men and 1.5 (95% CI 0.8-2.8, *p* trend=0.05) in women. RR comparing highest quintile to lowest quintile of milk intake was 1.8 (95% CI 1.2-2.9, *p* trend=0.02) in men and 1.5 (95% CI 0.8-2.8, *p* trend=0.05) in women. Analyses adjusted for age, smoking, energy intake, ibuprofen use, vigorous physical activity, educational level, and pesticide use

Table 2.4 (Continued)

Kyrozis, 2013	Cohort	15,063 women and 10,344 men from the European Prospective Investigation into Cancer and Nutrition	Total dairy, individual dairy foods	51 incident PD cases in women and 37 incident PD cases in men	RR was 1.33 (95% CI 1.07-1.65, *p* trend=0.009) for a 1 SD increase in total dairy and 1.34 (95% CI 1.14-1.58, *p* trend <0.001) for a 1 SD increase in milk, adjusting for gender, age, marital status, education, farming, smoking, coffee with caffeine, BMI, physical activity, and energy intake
Saaksjarvi, 2013	Cohort	2,136 women and 2,388 men from the Finnish Mobile Clinic Study	Individual dairy foods	40 incident PD cases in women and 45 incident PD cases in men	RR comparing highest tertile to lowest tertile was 1.24 (95% CI 0.48-3.17, *p* trend=0.87) in men and 3.31 (95% CI 1.10-9.93, *p* trend=0.09) in women, adjusting for age, marital status, community density, geographical area, smoking, body mass index, leisure-time physical activity, energy, hypertension, serum total cholesterol, diabetes, and, in women, parity

Table 2.5 Relative risk of PD by quintile of nutrient intake

	Quintile					P_{trend}
	1	2	3	4	5	
Dairy protein						
Women						
Median, gm	6.0	9.58	12.76	16.82	24.33	
Number of cases	87	87	97	109	101	
Relative risk (95% CI)						
Age-adjusted	1.0	0.92 (0.68, 1.24)	0.99 (0.74, 1.33)	1.09 (0.82, 1.44)	1.03 (0.77, 1.37)	p=0.49
Multivariate*	1.0	0.91 (0.67, 1.22)	0.96 (0.71, 1.28)	1.05 (0.78, 1.39)	0.98 (0.73, 1.31)	p=0.74
Men						
Median, gm	6.14	10.24	13.81	18.51	27.94	
Number of cases	87	104	116	131	116	
Relative risk (95% CI)						
Age-adjusted	1.0	1.16 (0.87, 1.54)	1.27 (0.96, 1.69)	1.44 (1.09, 1.89)	1.26 (0.95, 1.66)	p=0.07
Multivariate*	1.0	1.12 (0.84, 1.50)	1.23 (0.92, 1.62)	1.35 (1.02, 1.78)	1.18 (0.89, 1.56)	p=0.20
Pooled analysis						
Multivariate*	1.0	1.01 (0.82, 1.24)	1.09 (0.85, 1.38)	1.19 (0.93, 1.53)	1.08 (0.88, 1.32)	p=0.22
Total calcium						
Women						
Median, mg	471	621	766	994	1437	
Number of cases	59	114	105	112	91	
Relative risk (95% CI)						
Age-adjusted	1.0	1.63 (1.19, 2.24)	1.39 (1.01, 1.92)	1.43 (1.04, 1.96)	1.16 (0.83, 1.61)	p=0.74
Multivariate*	1.0	1.52 (1.11, 2.09)	1.26 (0.91, 1.75)	1.29 (0.94, 1.78)	1.05 (0.75, 1.47)	p=0.37
Men						
Median, mg	501	649	788	992	1418	
Number of cases	80	78	121	142	133	
Relative risk (95% CI)						

Table 2.5 (Continued)

Age-adjusted	1.0	0.88 (0.64, 1.20)	1.28 (0.96, 1.70)	1.45 (1.10, 1.91)	1.28 (0.96, 1.69)	P<0.01
Multivariate*	1.0	0.84 (0.61, 1.15)	1.19 (0.89, 1.59)	1.32 (1.00, 1.75)	1.19 (0.89, 1.58)	p=0.04
Pooled analysis						
Multivariate*	1.0	1.13 (0.63, 2.03)	1.22 (0.99, 1.52)	1.31 (1.06, 1.61)	1.13 (0.91, 1.40)	p=0.68
Dietary calcium						
Women						
Median, mg	444	562	666	795	1052	
Number of cases	76	97	102	107	99	
Relative risk (95% CI)						
Age-adjusted	1.0	1.11 (0.82, 1.51)	1.16 (0.86, 1.56)	1.17 (0.87, 1.57)	1.13 (0.83, 1.52)	p=0.47
Multivariate*	1.0	1.09 (0.81, 1.48)	1.13 (0.83, 1.52)	1.13 (0.84, 1.52)	1.07 (0.78, 1.45)	p=0.74
Men						
Median, mg	483	614	731	879	1207	
Number of cases	88	90	109	135	132	
Relative risk (95% CI)						
Age-adjusted	1.0	0.90 (0.67, 1.21)	1.10 (0.82, 1.45)	1.30 (0.99, 1.71)	1.28 (0.97, 1.68)	p<0.01
Multivariate*	1.0	0.87 (0.65, 1.18)	1.04 (0.78, 1.38)	1.19 (0.90, 1.57)	1.18 (0.90, 1.56)	p=0.04
Pooled analysis						
Multivariate*	1.0	0.97 (0.78, 1.21)	1.08 (0.88, 1.33)	1.16 (0.95, 1.42)	1.13 (0.92, 1.39)	p=0.08
Dairy calcium						
Women						
Median, mg	161.4	263.6	362.2	487.0	737.8	
Number of cases	78	100	90	108	105	
Relative risk (95% CI)						
Age-adjusted	1.0	1.18 (0.88, 1.59)	1.02 (0.75, 1.38)	1.20 (0.89, 1.60)	1.20 (0.90, 1.61)	p=0.23
Multivariate*	1.0	1.14 (0.85, 1.54)	0.98 (0.72, 1.32)	1.14 (0.85, 1.53)	1.12 (0.83, 1.51)	p=0.48
Men						
Median, mg	165.2	286.9	398.8	545.2	876.3	

Table 2.5 (Continued)

Number of cases	80	113	113	123	125	
Relative risk (95% CI)						
Age-adjusted	1.0	1.35 (1.01, 1.80)	1.29 (0.97, 1.73)	1.44 (1.09, 1.92)	1.44 (1.08, 1.91)	p=0.03
Multivariate*	1.0	1.34 (1.00, 1.79)	1.26 (0.94, 1.68)	1.36 (1.02, 1.81)	1.36 (1.02, 1.81)	p=0.09
Pooled analysis						
Multivariate*	1.0	1.24 (1.01, 1.53)	1.11 (0.87, 1.43)	1.24 (1.01, 1.53)	1.24 (1.01, 1.52)	p=0.08
Total vitamin D						
Women						
Median, IU	94.0	159.0	227.0	375.0	644.9	
Number of cases	43	111	121	110	96	
Relative risk (95% CI)						
Age-adjusted	1.0	2.12 (1.49, 3.02)	2.13 (1.50, 3.03)	1.91 (1.34, 2.72)	1.73 (1.21, 2.49)	p=0.22
Multivariate*	1.0	2.05 (1.44, 2.92)	2.01 (1.41, 2.86)	1.79 (1.25, 2.55)	1.65 (1.15, 2.38)	p=0.35
Men						
Median, IU	128.9	213.4	306.7	487.9	803.4	
Number of cases	73	112	108	133	128	
Relative risk (95% CI)						
Age-adjusted	1.0	1.33 (0.99, 1.79)	1.17 (0.87, 1.58)	1.33 (0.99, 1.77)	1.27 (0.95, 1.70)	p=0.26
Multivariate*	1.0	1.26 (0.94, 1.71)	1.08 (0.80, 1.46)	1.21 (0.90, 1.62)	1.15 (0.85, 1.54)	p=0.66
Pooled analysis						
Multivariate*	1.0	1.59 (0.99, 2.56)	1.46 (0.79, 2.70)	1.45 (0.99, 2.12)	1.35 (0.95, 1.94)	p=0.36
Dietary vitamin D						
Women						
Median, IU	83.5	128.9	170.3	219.4	314.3	
Number of cases	72	88	101	122	98	
Relative risk (95% CI)						
Age-adjusted	1.0	1.08 (0.79, 1.48)	1.20 (0.88, 1.62)	1.35 (1.01, 1.81)	1.14 (0.84, 1.56)	p=0.25
Multivariate*	1.0	1.06 (0.77, 1.44)	1.16 (0.85, 1.57)	1.29 (0.96, 1.74)	1.09 (0.80, 1.49)	p=0.42

Table 2.5 (Continued)

Men						
Median, IU	107.7	177.2	232.2	302.1	448.3	
Number of cases	81	103	88	155	127	
Relative risk (95% CI)						
Age-adjusted	1.0	1.15 (0.85, 1.54)	0.93 (0.68, 1.26)	1.60 (1.22, 2.10)	1.25 (0.94, 1.66)	p=0.02
Multivariate*	1.0	1.11 (0.83, 1.49)	0.89 (0.65, 1.20)	1.47 (1.12, 1.94)	1.14 (0.86, 1.52)	p=0.12
Pooled analysis						
Multivariate*	1.0	1.08 (0.88, 1.34)	1.01 (0.78, 1.32)	1.38 (1.13, 1.69)	1.12 (0.91, 1.38)	p=0.08
Dairy vitamin D						
Women						
Median, IU	16.1	35.2	67.9	108.0	195.4	
Number of cases	75	98	78	115	115	
Relative risk (95% CI)						
Age-adjusted	1.0	1.16 (0.86, 1.57)	0.89 (0.64, 1.22)	1.25 (0.94, 1.68)	1.28 (0.95, 1.71)	p=0.06
Multivariate*	1.0	1.12 (0.83, 1.52)	0.84 (0.61, 1.16)	1.19 (0.88, 1.60)	1.19 (0.88, 1.60)	p=0.17
Men						
Median, IU	17.3	44.0	81.6	128.6	245.4	
Number of cases	79	107	121	119	128	
Relative risk (95% CI)						
Age-adjusted	1.0	1.25 (0.93, 1.67)	1.32 (0.99, 1.76)	1.32 (0.99, 1.76)	1.37 (1.03, 1.82)	p=0.06
Multivariate*	1.0	1.22 (0.91, 1.64)	1.28 (0.96, 1.71)	1.23 (0.92, 1.64)	1.27 (0.95, 1.69)	p=0.25
Pooled analysis						
Multivariate*	1.0	1.17 (0.95, 1.45)	1.04 (0.69, 1.57)	1.21 (0.98, 1.48)	1.23 (1.00, 1.51)	p=0.08
Lactose						
Women						
Median, gm	2.37	5.37	9.51	14.53	25.35	
Number of cases	77	96	98	103	107	

Table 2.5 (Continued)

Relative risk (95% CI)						
Age-adjusted	1.0	1.13 (0.84, 1.53)	1.12 (0.83, 1.51)	1.13 (0.84, 1.53)	1.21 (0.90, 1.63)	p=0.26
Multivariate*	1.0	1.09 (0.80, 1.47)	1.06 (0.78, 1.43)	1.07 (0.79, 1.44)	1.12 (0.83, 1.51)	p=0.53
Men						
Median, gm	2.65	6.50	11.20	17.08	31.23	
Number of cases	82	101	122	117	132	
Relative risk (95% CI)						
Age-adjusted	1.0	1.10 (0.82, 1.47)	1.27 (0.96, 1.68)	1.23 (0.92, 1.64)	1.36 (1.03, 1.80)	p=0.02
Multivariate*	1.0	1.05 (0.78, 1.42)	1.20 (0.90, 1.59)	1.11 (0.83, 1.48)	1.23 (0.93, 1.64)	p=0.14
Pooled analysis						
Multivariate*	1.0	1.07 (0.86, 1.32)	1.13 (0.92, 1.39)	1.09 (0.88, 1.34)	1.18 (0.96, 1.45)	p=0.13

Using cumulative average intake levels.

*Adjusted for age, pack years of smoking, coffee intake, BMI, physical activity, alcohol intake, and total energy intake

Chapter 3 Genetic Variants Related to Urate and Risk of Parkinson's Disease

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ABSTRACT

Background: Higher urate concentrations have been associated with a lower risk of developing Parkinson's disease (PD) and with slower rates of clinical decline in PD patients. Whether these associations reflect a neuroprotective effect of urate is unclear.

Objective: To assess whether genetic variants associated with circulating urate levels are also associated with altered PD risk.

Methods: Analyses were conducted within three large ongoing cohort studies: the Nurses' Health Study (NHS), the Health Professionals Follow-up Study (HPFS), and the Cancer Prevention Study II Nutrition Cohort (CPS-IIN). We examined associations between single nucleotide polymorphisms (SNPs) in SLC2A9 and other genes involved in urate transport and PD risk using conditional logistic regression among 1,659 cases and 3,430 matched controls. We also used linear regression to assess associations between SNPs and plasma urate levels in a subset of 1,361 control participants with measured urate.

Results: We found the expected associations between SNPs in SLC2A9 and plasma urate levels among men and women; however, SNPs in other genes tended not to be associated with urate. Each SNP in SLC2A9 explained less than 7% of the variance in plasma urate. We did not find significant associations between the selected SNPs and PD risk among men or women.

Conclusion: Our results do not support an association between genetic variants associated with circulating urate levels and risk of PD.

INTRODUCTION

Urate is a powerful antioxidant that circulates at high concentrations in humans and is responsible for most of the antioxidant capacity in human plasma[1]. Because of the proposed role of oxidative stress in the pathology of Parkinson's disease, it has been suggested that high plasma urate levels could be protective against the disease[2,3]. Support for this hypothesis has come from a variety of sources. Laboratory models of PD have demonstrated that urate attenuates MPP+ toxicity in dopaminergic neurons[4] and 6-OHDA toxicity[5,6], and urate oxidase knock-out mice with increased concentrations of urate in the brain exhibit attenuated toxic effects of 6-OHDA on dopaminergic cells[7]. Several studies have reported that patients with PD have lower urate levels in serum[8-12] and plasma[13]. Prospective studies have shown that people with lower urate levels have an increased risk of developing PD[14-17]; in addition, higher serum and cerebrospinal fluid concentrations of urate have been associated with slower rates of clinical decline in PD patients[18-20]. However, several of the above studies reported associations only among men[12,14,15,17,18].

Evidence of an association between urate and PD suggests that urate could be a suitable target for neuroprotective therapies, since plasma urate levels can be increased through pharmacologic or dietary interventions. However, despite consistent evidence supporting an association between urate and PD, it is difficult to establish causality due to the inherent limitations of observational studies, particularly confounding. One approach to address this concern is to take advantage of genetic variants that affect urate levels to investigate causality. Since alleles are assigned randomly during meiosis, genotypes should be unrelated to confounding factors typical of epidemiologic studies. Several genetic loci have been associated with serum urate concentration in genome-wide association studies (GWAS), and one locus that has been consistently identified is *SLC2A9*[21-29]. *SLC2A9* encodes glucose transporter 9 (*GLUT9*), which can reabsorb urate in renal tubules[30]. Polymorphisms in *SLC2A9* have been associated

with age at onset of PD[31] as well as PD risk when combined with polymorphisms from other genes using a genetic score[32]. However, no association between 12 *SLC2A9* polymorphisms and PD risk was found in a separate study[33].

Therefore, we examined genetic variants that have previously been associated with altered urate levels in relation to PD risk among cases and controls selected from three large prospective cohort studies—the Nurses' Health Study (NHS), Health Professionals Follow-up Study (HPFS), and the Cancer Prevention Study II Nutrition Cohort (CPS-IIN). In addition, as detailed exposure histories have been collected from all members of these cohorts as well as measured plasma urate for a subset of participants, we have a unique ability to assess relationships among genetic variants, plasma urate, and lifestyle factors.

METHODS

Study population

The NHS was established in 1976 when 121,700 female registered nurses aged 30 to 55 years completed a mailed questionnaire regarding their medical histories and baseline health-related exposures. The HPFS was established in 1986 when 51,529 male health professionals aged 40 to 75 years responded to a similar questionnaire. The CPS-IIN, a sub-cohort of the larger CPS-II, includes 184,190 individuals (86,404 men and 97,786 women) aged 50 to 74 in 1992 who completed a questionnaire regarding nutrition and other risk factors. For all cohorts, follow-up questionnaires have been sent every two years to collect updated exposure data and disease diagnoses. Follow-up in all cohorts has been approximately 90% or higher.

Case ascertainment and control selection

In all cohorts, cases have been identified through biennial self-report questionnaires. When a diagnosis of PD was reported, we asked the treating neurologist to complete a questionnaire to confirm the diagnosis or to send a copy of the medical records. Prior to 2003, cases were confirmed if the diagnosis was considered definite or probable by the treating neurologist or internist, or if review of medical records by investigators blind to exposure status revealed a final diagnosis of PD by a neurologist or evidence at a neurologic assessment of at least two of the four cardinal signs of PD (with one being resting tremor or bradykinesia), a progressive course, and the absence of features suggesting an alternative diagnosis. For cases of PD reported since 2003, the above procedure was used with the exception that medical records were requested from all cases and were reviewed by a neurologist specializing in movement disorders. If the determination of the movement disorders specialist conflicted with that of the neurologist, the decision of the movement disorders specialist was used. The validity of this case ascertainment method is supported by previous validation studies in which neurologists of confirmed PD cases were re-contacted several years after their patients' initial diagnosis. It was found that 96% of cases previously considered to be definite or probable were confirmed to have PD after reviewing their update medical records, while 4% were thought to be uncertain cases.

For each case, we randomly selected controls who were alive and had not reported PD at the time of the case's diagnosis. We selected between 2 and 6 controls per case in the NHS and HPFS and one control per case in the CPS-IIN cohort. Controls were matched to the cases based on cohort, sex, birth year (+/- 1 year), race (white vs. other), fasting status (>8 hours vs. less/unknown), and year, month, and time of blood draw (in two-hour intervals).

Plasma urate assessment

Participants from each cohort were invited to provide blood samples for the purpose of investigating biomarkers of chronic diseases. Blood samples were collected from 32,826 members of NHS between 1989 and 1990, 18,000 members of HPFS between 1993 and 1995, and 40,000 members of CPS-IIN between 1998 and 2001. For NHS and HPFS, subjects used collection kits that were provided to them by the studies and sent blood samples via overnight delivery to our lab. More than 95% of samples were delivered within 26 hours of being drawn and approximately 75% were drawn at least 8 hours after the participant's last meal. Upon arrival, blood samples were centrifuged and blood components were aliquoted into cryotubes and stored in the vapor phase of liquid nitrogen freezers at -130 degrees C or colder until being sent to the laboratory for analysis. For CPS-IIN, participants went to participating hospitals in their communities for blood draws. Hospital staff centrifuged the samples to separate blood components prior to shipping. They then shipped samples overnight to a central repository where the samples were aliquoted and frozen in the vapor phase of liquid nitrogen freezers for long-term storage[34]. Samples from cases and controls were handled identically. Concentrations of plasma urate were measured using a colorimetric enzyme assay (Hitachi 911; Roche Diagnostics, Indianapolis, Indiana). Coefficients of variation (CVs) were determined using blinded quality control samples included with the study samples. All reported CVs were less than 10%.

Genotyping

Genotyping was conducted for 332 confirmed cases and 1243 controls in NHS, 358 confirmed cases and 1218 controls in HPFS, and 969 cases and 969 controls in CPS-IIN (the 969 cases in CPS-IIN included 371 individuals whose medical records were incomplete or could not be obtained; these individuals were excluded in sensitivity analyses). Genotyping was carried out through the Harvard Partners Center for Genetics and Genomics at the Harvard Partners Genotyping Facility using the

OpenAssay SNP Genotyping System (BioTrove, Woburn, Massachusetts, USA). Our primary gene of interest, *SLC2A9*, has been identified in several GWA studies as the strongest genetic predictor of serum urate levels and gout[21-24,35,36]. Although the causal variant has not been identified, we genotyped three SNPs due to their strong associations with urate in previous GWAS: rs6855911[21,24,27], located within intron 7 with minor allele frequency (MAF) of 0.31 (G allele); rs7442295[21,22,24,27], located within intron 6 with a MAF of 0.21 for G allele; and rs16890979[23,26,27], a missense mutation in exon 8 with a MAF of 0.22 for T allele (using HapMap data from Utah residents with ancestry from northern and western Europe, abbreviated CEU[37]). These three SNPs are in strong linkage disequilibrium (LD; pairwise r^2 range from 0.68-0.76 from Haploview[38] with HapMap CEU data) and each minor allele of these SNPs has been associated with a 0.30-0.43 mg/dL decrease in serum urate in individuals of European descent[21,23]. In addition to SLC2A9, we selected for analysis other genes of interest due to their role in the transport of urate, including solute carrier family 22, member 12 (*URAT1/SLC22A12)*, ATP-binding cassette sub-family G member 2 *(ABCG2)*, and solute carrier family 19 (sodium phosphate), member 3 *(SLC17A3)*.

Covariate assessment

Data on other covariates, including age, weight, height, smoking, physical activity, usual diet, and medications, were collected via self-report questionnaires every two years, as previously described[39]. Body mass index (BMI) was calculated as weight(kg)/height(m)^2.

Statistical analyses

Basic characteristics of the study population were assessed using means for continuous variables and percentages for discrete variables. Given previously reported sex differences for the association between urate and PD risk, we performed analyses separately in men and women. We used histograms and q-q plots to check for normality and then examined associations between individual

SNPS and urate using linear regression under an additive genetic model for individuals with measured urate. We used R^2 as a measure of the proportion of the variation in plasma urate explained by each SNP. Because only the SNPs located within SLC2A9 demonstrated statistically significant associations with urate, we also created a genetic score by summing the number of SLC2A9 minor alleles that have been associated with lower urate in previous GWAS. Finally we explored possible modification of the association between the genetic score and urate by BMI, the dietary urate index[39], and the components of the dietary urate index: vitamin C, fructose, alcohol, and dairy protein, by including cross-product terms between these variables (higher vs. lower, based on the median values) and the genetic score. We then conducted analyses of the genetic score and urate within levels of variables identified as effect modifiers through testing of the interaction terms.

We then assessed the association between each SNP and PD risk under an additive genetic model using conditional logistic regression. In a second model we additionally adjusted for smoking status, coffee intake, body mass index, and alcohol consumption. Covariates were obtained from the questionnaire preceding blood draw. In addition to the individual SNPs, we also examined the association between the genetic score and PD using conditional logistic regression. We performed analyses first separately by study and gender, and then used a random meta-analysis approach to pool results and assess potential heterogeneity. Finally, we estimated the association between genetically determined plasma urate and PD risk using the two-stage regression described below. First we fit a linear regression model within the subset of participants with measured urate and SNPs, with plasma urate as the dependent variable and the three SLC2A9 SNPs as independent variables. Then we used the genetically determined urate levels generated in the first stage as a continuous independent variable in a conditional logistic regression model for PD. All statistical analyses were conducted using SAS (SAS Institute, Cary, NC).

RESULTS

Baseline characteristics are presented in Table 3.1. A total of 1678 cases and 4052 controls were included. We examined associations between SNPs and urate in a subset of 1361 controls with measured urate. As expected, urate levels were higher among men than women. In both men and women across all three cohorts, we found statistically significant associations between all three SLC2A9 SNPs and urate, with each explaining 4.30-5.46% of the overall variance in plasma urate among women and 3.12-4.29% among men (Figure 3.1). RS6855911 exhibited the strongest associations with plasma urate among women--a one-allele increment was associated with a 0.47 mg/dL decrease in urate levels. Among men, rs7442295 was most strongly associated with plasma urate, and a one-allele increment was associated with a 0.40 mg/dL decrease in urate levels. Associations were similar in men and women. The genetic score consisting of all three SLC2A9 SNPs explained 5.19% of the variation in urate levels in women and 3.90% in men. SNPs from other genes were not associated with urate levels (Appendix Table 3.3) and did not contribute to explaining more of the variation—for example, an alternative genetic score that included all measured SNPs explained 3.19% of the variation in urate levels in women and 2.54% in men.

Table 3.1 Age-adjusted characteristics of the study population at time of blood draw

	Women		Men	
	Controls (n=2151)	Cases (n=650)	Controls (n=1901)	Cases (n=655)
Age, years*	60.2(6.4)	60.8(6.5)	65.1(6.8)	65.1(6.6)
Urate, mg/dL	4.7(1.2)	4.7(1.2)	5.7(1.5)	5.5(1.1)
Body mass index, kg/m^2	26.4(5.1)	26.3(4.4)	25.6(3.7)	25.2(2.8)
Current smoker, %	11	7	5	2
Past smoker, %	38	33	56	52
Caucasian, %	98	98	99	99
Alcohol, g/day	4.9(9.9)	3.4(6.6)	10.7(16.3)	9.2(14.3)
Coffee, servings/day	1.6(1.7)	1.6(1.7)	1.8(1.8)	1.7(1.9)

Values are means(SD) or percentages and are standardized to the age distribution of the study population.
* Value is not age adjusted

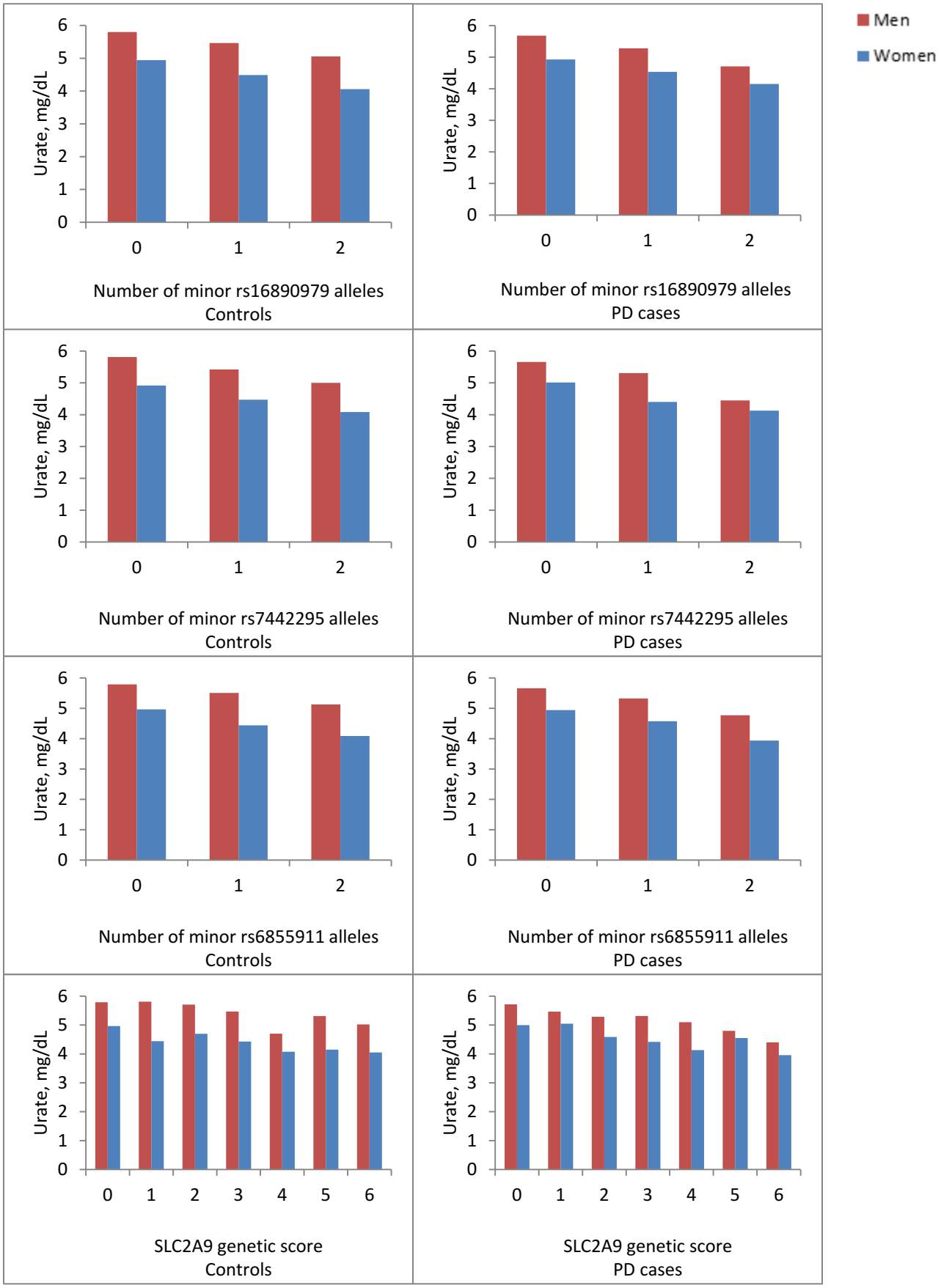

Figure 3.1 Plasma urate level by number of SLC2A9 variant alleles in PD cases and controls. *$p_{trend} < 0.05$, **$p_{trend} < 0.001$, *$p_{trend} < 0.0001$**

We then examined associations between each SNP and risk of PD. Results from minimally and fully adjusted models did not show significant associations with PD risk in men or women (Table 3.2). Pooled, multivariable-adjusted odds ratios ranged from 0.89 to 1.10, with 95% confidence intervals all including 1.0. The genetic score including the three SNPs within SLC2A9 was also not associated with PD risk: the pooled RR was 1.00 (0.94, 1.05). Finally, we performed a two-stage regression to examine the association between genetically-predicted urate and PD risk. Consistent with the results above, we did not find significant associations for men (pooled RR for a 1mg/dL higher urate = 0.95, 95% CI 0.63-1.43) or women (pooled RR for a 1mg/dL higher urate 1.10, 95% CI 0.76-1.57). Results did not change after excluding cases without confirmation from medical records or after excluding individuals whose PD was diagnosed after age 80. In exploratory analyses, we investigated the association between SNPs and urate stratified by body mass index and the dietary urate index and its components. We found an interaction between fructose and SLC2A9 variants among men (*p* for interaction=0.002), where the association between additional variants and lowered urate was only apparent for men below the median fructose intake. For those with intake below the median level, each additional minor allele was associated with 0.23 mg/dL lower urate (95% CI -0.32, -0.15), while for those above the median level each additional minor allele was associated with only 0.03 mg/dL lower urate (95% CI -0.11, 0.05). No other significant interactions were found (p>0.05 for all). Fructose intake has been associated with higher serum urate levels among men, but not women[40]. SLC2A9-mediated urate transport is facilitated by fructose, which might partly explain this result[41]. However, SLC2A9 variants were still not associated PD risk among men with low fructose intake (RR=0.97, 95% CI 0.77-1.21).

Table 3.2 Associations between genetic variants and PD risk

	Women		Men		Pooled
	RR (95% CI)	RR (95% CI), fully adjusted*	RR (95% CI)	RR (95% CI), fully adjusted*	RR (95% CI), fully adjusted*
N cases, controls	628, 1641		813, 1700		
SLC2A9					
rs16890979	0.96 (0.82, 1.14)	0.86 (0.65, 1.14)	1.04 (0.89, 1.21)	1.05 (0.89, 1.24)	0.98 (0.85, 1.13)
rs7442295	0.99 (0.83, 1.17)	0.87 (0.58, 1.29)	1.00 (0.86, 1.17)	1.02 (0.86, 1.22)	0.97 (0.81, 1.16)
rs6855911	0.97 (0.83, 1.14)	0.92 (0.74, 1.14)	1.06 (0.92, 1.23)	1.09 (0.93, 1.29)	1.03 (0.90, 1.17)
SNP score	0.99 (0.93, 1.05)	0.95 (0.86, 1.06)	1.02 (0.96, 1.07)	1.02 (0.96, 1.08)	1.00 (0.94, 1.05)
URAT1					
rs11231825	1.04 (0.90, 1.21)	1.13 (0.87, 1.45)	1.06 (0.73, 1.54)	1.05 (0.67, 1.62)	1.10 (0.89, 1.35)
rs11602903	0.98 (0.85, 1.14)	1.06 (0.87, 1.30)	1.07 (0.77, 1.47)	1.02 (0.67, 1.55)	1.06 (0.88, 1.28)
rs3825016	1.01 (0.87, 1.17)	1.08 (0.89, 1.32)	1.06 (0.77, 1.47)	1.07 (0.76, 1.52)	1.10 (0.94, 1.28)
rs3825018	0.96 (0.82, 1.12)	0.91 (0.68, 1.23)	0.93 (0.74, 1.16)	0.96 (0.70, 1.30)	0.93 (0.78, 1.10)
rs475688	0.97 (0.80, 1.18)	0.91 (0.67, 1.23)	0.92 (0.72, 1.17)	0.93 (0.70, 1.22)	0.91 (0.78, 1.08)
rs476037	1.01 (0.76, 1.34)	1.09 (0.71, 1.68)	1.08 (0.71, 1.65)	0.95 (0.46, 1.94)	1.03 (0.73, 1.45)
rs7932775	1.08 (0.91, 1.29)	1.19 (0.79, 1.78)	1.01 (0.85, 1.20)	1.02 (0.84, 1.24)	1.08 (0.91, 1.27)
rs893006	0.98 (0.84, 1.14)	0.93 (0.76, 1.13)	0.93 (0.75, 1.14)	0.94 (0.72, 1.22)	0.92 (0.80, 1.05)
ABCG2					
rs2231142	0.81 (0.64, 1.02)	0.79 (0.59, 1.08)	1.28 (1.01, 1.60)	1.19 (0.81, 1.75)	0.98 (0.72, 1.33)
SLC17A3					
rs1165205	0.90 (0.76, 1.06)	0.89 (0.74, 1.06)	0.90 (0.78, 1.03)	0.89 (0.76, 1.03)	0.89 (0.79, 1.00)

*Adjusting for matching factors and BMI, alcohol intake, caffeine intake, and smoking status

DISCUSSION

Previous research has demonstrated an association between urate and lowered PD risk; however, whether this association reflects a neuroprotective effect of urate is difficult to confirm in observational studies because of the possibility of unmeasured confounding. Since polymorphisms should be minimally confounded by other factors, studying the association between genetic determinants of urate levels and PD risk could provide important insights into the relationship between urate and PD. In this analysis, we found the expected associations between variants in SLC2A9 and urate levels among both men and women in three cohorts; however, we did not find associations between these genetic variants and PD risk.

One explanation of our results is that the relatively small proportion of the variation in urate levels explained by the SNPs in this analysis mean that the association between urate and PD cannot be detected using these variants. Since genetic effects on phenotypes are typically small, this is a common drawback to Mendelian randomization studies. Since the SLC2A9 score only accounted for 5.19% of the variation in urate concentrations in women and 3.90% in men, genetic factors other than the common variants assessed in this analysis may contribute more to between-person differences in circulating urate levels. In a recent analysis in these cohorts, we found approximately a 40% reduced risk of PD among men in the highest quartile of plasma urate compared to men in the lowest quartile[42]. Based on results from a meta-analysis[43], we estimated that the approximately 0.80mg/dL decrease in serum urate associated with having two copies of the variant allele compared to having none for one of the SNPs in SLC2A9 would predict a 14.5% increase in PD risk. The power of our study may thus have been insufficient to detect this relatively modest difference in genetically determined PD risk.

Another possible explanation of our results is that plasma urate does not have a causal effect on PD. While possible, these findings should be weighed against the evidence from a variety of sources

supporting the hypothesis that urate is neuroprotective. Higher urate levels have been associated with decreased risk of PD in prospective studies[12-15] and with slower rates of clinical decline in PD patients[16,17]. Dietary determinants of urate have also been associated with altered PD risk[39]. Of note, a recent analysis using the same SLC2A9 variants found a hazard ratio for disease progression of 1.27 for a 0.5mg/dL genetically conferred decrease in serum urate[44], which may suggest that urate is a stronger predictor of PD progression than risk. Further, it is possible that genetic determinants of plasma urate differ from genetic determinants of CNS urate. While cerebrospinal fluid, brain, or neuronal urate may be neuroprotective, as plasma urate does not directly determine urate levels in the immediate environment of the degenerating neurons, it may be unrelated or only weakly related to PD risk.

The strengths of our study include the relatively large sample size and the availability of genetic data as well as plasma urate measurements and data on diet and lifestyle factors. One weakness of our study is that the participants are almost exclusively of European descent, which limits the generalizability of our findings. However, restricting our analysis to individuals of European descent also minimizes the potential for confounding by population stratification. In addition, for SLC2A9 in particular, associations with urate levels have been found in many different populations[21-29].

In conclusion, our results do not support an association between genetic variants associated with circulating urate levels and risk of PD, but larger investigations are needed to determine whether the modest genetic effects on blood urate contribute to predict PD risk.

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APPENDIX

Table 3.3 Association between genetic variants and uric acid among controls

	Men						Women					
	HPFS (N=248)			CPS (N=374)			NHS (N=509)			CPS (N=230)		
	R^2 (%)	Beta*	95% CI	R^2 (%)	Beta*	95% CI	R^2 (%)	Beta*	95% CI	R^2 (%)	Beta*	95% CI
SLC2A9												
rs16890979	6.55	-0.46	(-0.69, -0.24)	1.89	-0.27	(-0.46, -0.07)	5.33	-0.48	(-0.66, -0.30)	4.16	-0.43	(-0.71, -0.16)
rs7442295	5.20	-0.44	(-0.69, -0.20)	2.76	-0.34	(-0.55, -0.13)	4.23	-0.43	(-0.61, -0.25)	3.72	-0.42	(-0.70, -0.14)
rs6855911	3.60	-0.33	(-0.56, -0.11)	2.11	-0.28	(-0.47, -0.08)	4.99	-0.47	(-0.65, -0.28)	4.14	-0.43	(-0.70, -0.16)
Score	5.43	-0.16	(-0.22, -0.09)	2.42	-0.11	(-0.18, -0.04)	5.70	-0.19	(-0.25, -0.13)	4.19	-0.15	(-0.25, -0.06)
URAT1												
rs11231825	0.03	-0.03	(-0.25, 0.19)	2.60	-0.30	(-0.49, -0.11)	0.40	0.13	(-0.05, 0.30)	0.41	-0.12	(-0.12, 0.36)
rs11602903	0.04	0.03	(-0.20, 0.26)	2.40	-0.29	(-0.49, -0.10)	0.55	0.15	(-0.03, 0.32)	0.49	0.13	(-0.11, 0.37)
rs3825016	0.07	-0.04	(-0.27, 0.18)	2.49	-0.30	(-0.49, -0.11)	0.34	0.12	(-0.06, 0.29)	0.39	0.12	(-0.13, 0.36)
rs3825018	0.01	-0.02	(-0.26, 0.22)	2.69	0.31	(0.12, 0.51)	0.34	-0.11	(-0.28, 0.06)	0.53	-0.13	(-0.37, 0.11)
rs475688	0.58	-0.13	(-0.35, 0.09)	2.81	0.34	(0.13, 0.55)	0.01	-0.02	(-0.22, 0.17)	1.09	-0.20	(-0.45, 0.05)
rs476037	0.12	0.10	(-0.29, 0.49)	0.66	-0.24	(-0.54, 0.06)	0.20	0.13	(-0.13, 0.38)	0.05	0.06	(-0.29, 0.40)
rs7932775	0.54	-0.15	(-0.42, 0.12)	2.34	-0.33	(-0.54, -0.11)	0.07	0.06	(-0.15, 0.27)	0.10	0.07	(-0.21, 0.35)
rs893006	0.05	0.04	(-0.19, 0.27)	1.99	0.27	(0.07, 0.46)	0.23	-0.10	(-0.28, 0.08)	0.15	-0.07	(0.31, 0.17)
ABCG2												
rs2231142	0.83	0.22	(-0.08, 0.53)	0.04	0.07	(-0.27, 0.40)	0.06	0.09	(-0.22, 0.39)	1.01	0.29	(-0.09, 0.66)
SLC17A3												
rs1165205	1.30	-0.18	(-0.38, 0.02)	0.03	-0.03	(-0.20, 0.14)	0.19	-0.08	(-0.25, 0.09)	1.84	-0.23	(-0.45, -0.01)

*Represents difference in urate in mg/dL for each additional minor allele